

=> d his

(FILE 'HOME' ENTERED AT 06:51:51 ON 21 APR 2005)
DEL HIS

FILE 'HCAPLUS' ENTERED AT 06:53:57 ON 21 APR 2005
L1 712 S ?PROUK? OR ?PROUROKINASE? OR PRO() (UK OR UROKINASE)
L2 3 S RPRO() (UK OR UROKINASE)
L3 712 S L1,L2

FILE 'REGISTRY' ENTERED AT 06:55:45 ON 21 APR 2005
L4 1 S 82657-92-9
E PROUROKINASE
L5 151 S E3

FILE 'HCAPLUS' ENTERED AT 06:57:31 ON 21 APR 2005
L6 684 S L4
L7 714 S L5
L8 545 S ABT 187 OR ABT187 OR PRO U PA OR PROLYSE OR PROLYZE OR PUK OR
L9 1171 S L3,L6-L8
L10 7 S PET29A
L11 20 S ?PET29?
L12 4 S L9 AND L10,L11
L13 9 S L9 AND T7
L14 6 S L9 AND (SHINE OR DALGARNO)
L15 16 S L9 AND (BL21 OR DE3 OR RIL)
L16 7 S L12-L14 AND L15
E E COLI/CT
E ESCHERICHIA/CT
L17 148991 S E3+OLD,NT,PFT,RT OR E14+OLD,NT,PFT,RT
L18 248421 S ("E" OR ESCHERICH?) ()COLI
L19 176 S L9 AND L17,L18
L20 2 S L19 AND TYPE B
L21 108 S L9 AND B
L22 3 S L20,L21 AND L10-L16
L23 153 S L9 AND ESCHERI?
L24 21 S L23 AND L10-L16
L25 3 S L24 AND (TYPE B OR B)
L26 27 S L12-L16,L20,L22,L25
L27 235 S L19,L21,L23,L24 NOT L26
L28 4 S L26 AND ?MUTANT?
L29 2 S L26 AND ?MUTAT?
L30 36 S L27 AND (?MUTANT? OR MUTAT?)
L31 2 S L26 AND ?MUTAGEN?
L32 15 S L27 AND ?MUTAGEN?
L33 4 S L28,L29,L31
L34 41 S L30,L32 NOT L33
L35 2 S L9 AND FLEX?(L) LOOP?
L36 1 S L9 AND WOBBL?(L) LOOP?
L37 2 S L35,L36
L38 6 S L9 AND (LYS300 OR LYS 300)
L39 0 S L9 AND (LYSINE300 OR LYSINE 300)
L40 27 S L9 AND (HIS OR HISTID?)
L41 124 S L9 AND (LYS OR LYSINE OR LYSYL?)
L42 12 S L40 AND L41

FILE 'REGISTRY' ENTERED AT 07:06:40 ON 21 APR 2005
L43 1 S 56-87-1
L44 3 S (D-LYSINE OR DL-LYSINE)/CN OR L43
L45 3 S (L-HISTIDINE OR D-HISTIDINE OR DL-HISTIDINE)/CN

FILE 'HCAPLUS' ENTERED AT 07:07:20 ON 21 APR 2005
L46 24 S L44 AND L9

L47 6 S L45 AND L9
 L48 13 S L38,L41,L46 AND L40,L47
 L49 2 S L48 AND L10-L16,L26
 L50 4 S L48 AND L27
 L51 3 S L48 AND L28-L37
 L52 6 S L49-L51
 L53 5 S L52 NOT CRYOGEL/TI
 L54 96 S L10-L16,L20,L22,L25,L26,L28-L37,L48
 L55 5 S L54 AND L53
 L56 4 S L55 NOT MMP 3/TI
 L57 91 S L54 NOT L55
 L58 15 S L57 AND PROUROKINASE/TI
 L59 19 S L56,L58
 L60 76 S L57 NOT L59
 L61 35 S L60 AND L6
 L62 38 S L60 AND L7
 L63 57 S L61,L62,L59
 L64 38 S L54 NOT L55-L56,L58,L59,L61-L63
 SEL DN AN 13-16 22 24 26 29-34 36-38
 L65 16 S L64 AND E1-E48
 L66 73 S L63,L65
 L67 72 S L66 AND ?UROKINASE?
 L68 1 S L66 NOT L67
 E SARMIENTOS P/AU
 L69 46 S E3,E4
 E PAGANI M/AU
 L70 45 S E3-E8,E18
 L71 9 S L69,L70 AND L9
 L72 12 S L69,L70 AND ?UROKINASE?
 L73 12 S L71,L72
 L74 2 S US20050019863/PN OR (WO2004-US11840 OR US2004-826598# OR US20
 L75 1 S L74 AND L9
 L76 1 S L74 AND ?UROKINASE?
 L77 12 S L75,L76,L73
 L78 78 S L77,L67
 L79 78 S L78 AND L1-L3,L6-L42,L46-L78
 L80 75 S L79 AND (PD<=20030418 OR PRD<=20030418 OR AD<=20030418)
 L81 3 S L79 NOT L80
 L82 78 S L79-L80
 L83 49 S L82 AND (?MUTANT? OR ?MUTAGEN? OR ?MUTAT?)
 E MUTANT/CT
 E MUTAT/CT
 L84 332100 S E7+OLD,NT,PFT,RT
 E MUTAGEN/CT
 L85 202397 S E5+OLD,NT,PFT,RT OR E5-E10
 L86 268465 S E16+OLD,NT,PFT,RT
 L87 13 S L82 AND L84-L86
 L88 50 S L83,L87
 L89 45 S L82 AND (RECOMBIN? OR CHIMER?)
 E RECOMBINANT/CT
 L90 0 S L82 AND E11+OLD,NT,PFT,RT
 L91 0 S L82 AND E41+OLD,NT,PFT,RT
 L92 28 S L82 AND E48+OLD,NT,PFT,RT
 E E48+ALL
 L93 28 S L82 AND E9+OLD,NT,PFT,RT
 L94 4 S L82 AND E7+OLD,NT,PFT,RT
 L95 70 S L88-L94
 L96 8 S L82 NOT L95
 SEL DN AN 1 4 5
 L97 5 S L96 NOT E1-E9
 L98 75 S L95,L97

=> fil reg

FILE 'REGISTRY' ENTERED AT 07:29:12 ON 21 APR 2005
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2005 American Chemical Society (ACS)

Property values tagged with IC are from the ZIC/VINITI data file
provided by InfoChem.

STRUCTURE FILE UPDATES: 20 APR 2005 HIGHEST RN 848887-73-0
DICTIONARY FILE UPDATES: 20 APR 2005 HIGHEST RN 848887-73-0

New CAS Information Use Policies, enter HELP USAGETERMS for details.

TSCA INFORMATION NOW CURRENT THROUGH JANUARY 18, 2005

Please note that search-term pricing does apply when
conducting SmartSELECT searches.

*
* The CA roles and document type information have been removed from *
* the IDE default display format and the ED field has been added, *
* effective March 20, 2005. A new display format, IDERL, is now *
* available and contains the CA role and document type information. *
*

Crossover limits have been increased. See HELP CROSSOVER for details.

Experimental and calculated property data are now available. For more
information enter HELP PROP at an arrow prompt in the file or refer
to the file summary sheet on the web at:
<http://www.cas.org/ONLINE/DBSS/registryss.html>

=> d ide can 14

L4 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2005 ACS on STN
RN 82657-92-9 REGISTRY
ED Entered STN: 16 Nov 1984
CN Kinase (enzyme-activating), prouro- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN ABT-187
CN Pro u-PA
CN Pro-UK
CN Pro-urokinase-type plasminogen activator
CN Prolyse
CN Prourokinase
CN Prourokinase plasminogen activator
CN PUK
CN Scu-PA
CN Single-chain pro-urokinase
CN Single-chain urokinase
CN Single-chain urokinase-type plasminogen activator
CN Thombolyse
CN Tomizeze
MF Unspecified
CI COM, MAN
LC STN Files: ADISINSIGHT, ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS,
BIOTECHNO, CA, CAPLUS, CBNB, CIN, CSCHEM, DDFU, DRUGU, EMBASE, IPA,
MRCK*, PHAR, PROMT, PROUSDDR, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

682 REFERENCES IN FILE CA (1907 TO DATE)
70 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
684 REFERENCES IN FILE CAPLUS (1907 TO DATE)

REFERENCE 1: 142:316831
REFERENCE 2: 142:293700
REFERENCE 3: 142:293158
REFERENCE 4: 142:285326
REFERENCE 5: 142:255442
REFERENCE 6: 142:237004
REFERENCE 7: 142:211364
REFERENCE 8: 142:109438
REFERENCE 9: 142:11628
REFERENCE 10: 141:423372

=> fil hcaplus
FILE 'HCAPLUS' ENTERED AT 07:29:23 ON 21 APR 2005
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

Copyright of the articles to which records in this database refer is held by the publishers listed in the PUBLISHER (PB) field (available for records published or updated in Chemical Abstracts after December 26, 1996), unless otherwise indicated in the original publications. The CA Lexicon is the copyrighted intellectual property of the American Chemical Society and is provided to assist you in searching databases on STN. Any dissemination, distribution, copying, or storing of this information, without the prior written consent of CAS, is strictly prohibited.

FILE COVERS 1907 - 21 Apr 2005 VOL 142 ISS 17
FILE LAST UPDATED: 20 Apr 2005 (20050420/ED)

New CAS Information Use Policies, enter HELP USAGETERMS for details.

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> d 198 bib abs hitrn retable tot

L98 ANSWER 1 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
AN 2004:936297 HCAPLUS
DN 142:255442
TI Construction, expression, and characterization of a recombinant annexin B1-low molecular weight urokinase chimera in *Escherichia coli*
AU Yan, Hong-Li; Wang, Wei-Ting; He, Yan; Zhao, Zhuan-You; Gao, Yuan-Jian; Zhang, Yi; Sun, Shu-Han
CS Department of Medical Genetics, Second Military Medical University, Shanghai, 200433, Peop. Rep. China
SO *Acta Biochimica et Biophysica Sinica* (2004), 36(3), 184-190

Goldstein

PB CODEN: ABBSC2; ISSN: 1672-9145
 DT Shanghai Scientific and Technical Publishers
 LA Journal
 English
 AB To produce a thrombi-targeting plasminogen activator, low mol. weight single-chain urokinase gene (scuPA32k) was spliced with the full-length cDNA of annexin B1 gene (anxB1) by overlap extension method. The fused gene anxB1scuPA was ligated into pET28a vector, transformed into *E. coli* BL21-RIL, and then induced to express under the control of T7 promoter. The AnxB1 ScuPA protein expressed amounted to 22% of the total bacterial proteins. The product was refolded, and then purified by using DEAE Sepharose fast flow ion-exchange column and Superdex S-200 gel-filtration column. HPLC anal. revealed that the final purity is about 95%. The specific activity of AnxB1ScuPA, measured as amidolytic activity, reached 100,000 IU/mg. It had a similar S2444 catalytic efficiency (kcat/Km) to ScuPA32k, and also showed high activated-platelet membrane-binding activity and anticoagulant activity, indicating that the chimera fully retained the components of enzymic and membrane-binding activities of the parent mols. In vivo test revealed that, the dogs administered with AnxB1ScuPA had less reperfusion time, higher reperfusion ratio, and less bleeding effects than those with urokinase. These findings indicated that AnxB1ScuPA might have advantages over current available thrombolytic agents.

IT 82657-92-9, Single-chain urokinase
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (construction, expression, and characterization of a recombinant annexin B1-low mol. weight urokinase chimera in *Escherichia coli*)

RETABLE

Referenced Author (RAU)	Year (R PY)	VOL (R VL)	PG (R PG)	Referenced Work (RWK)	Referenced File
Bradford, M	1976	72	248	Anal Biochem	HCAPLUS
Cahill, M	1993	50	221	Br J Biomed Sci	HCAPLUS
Deckmyn, H	1994	87	562	Br J Haematol	HCAPLUS
Ho, S	1989	77	51	Gene	HCAPLUS
Laemmli, U	1970	227	680	Nature	
Lenich, C	1992	68	539	Thromb Haemost	HCAPLUS
Lu, S	1999		380	Current Protocols fo	
Marshak, D	1996		12	Strategies for Prote	
Sambrook, J	2001		66	Molecular Cloning:A	
Tanaka, K	1996	35	922	Biochemistry	HCAPLUS
Vrkljan, M	1994	11	1004	Pharm Res	HCAPLUS
Yan, H	2002	119	1	Mol Biochem Parasito	HCAPLUS
Yan, H	2004	25	54	Science in China	
Zhang, Y	2002	23	378	Science in China	
Zhao, Z	2001	37	420	Journal of Nanjing U	HCAPLUS

L98 ANSWER 2 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2004:927004 HCAPLUS

DN 141:378919

TI Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound-sealing clots

IN Gurewich, Victor; Williams, John N.; Liu, Jian-Ning; Sarmientos, Paolo; Pagani, Massimiliano

PA Thrombolytic Science, Inc., USA

SO PCT Int. Appl., 90 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.

KIND DATE

APPLICATION NO.

DATE

PI WO 2004093797 A2 20041104 WO 2004-US11840 20040416 <--
 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
 CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
 GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
 LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI,
 NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
 TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
 RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ,
 BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE,
 ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
 SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN,
 TD, TG
 CA 2426115 AA 20041018 CA 2003-2426115 20030422 <--
 US 2005019863 A1 20050127 US 2004-826598 20040416 <--
 US 2005031607 A1 20050210 US 2004-826826 20040416 <--

PRAI US 2003-463930P P 20030418 <--
 US 2003-464002P P 20030418 <--
 US 2003-464003P P 20030418 <--

AB It has now been discovered that various **mutant** forms of **pro-urokinase** ('**pro-UK**') perform in the manner of **pro-UK** in lysing 'bad' blood clots (those clots that occlude blood vessels), while sparing hemostatic fibrin in the so-called 'good' blood clots (those clots that seal wounds, e.g., after surgery or other tissue injury). Thus, these **pro-UK mutants** are excellent and safe thrombolytic agents. These advantages allow them to be used in a variety of new methods, devices, and compns. useful for thrombolysis and treating various cardiovascular disorders in clin. situations where administration of other known thrombolytic agents has been too risky or even contraindicated. New methods of making the **pro-UK mutants** are also disclosed.

IT 82657-92-9P, **Prourokinase**
 RL: BPN (Biosynthetic preparation); PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
 (activated two-chain **mutant**; **mutant** **prourokinase** compns. for lysis of occlusive blood clots while sparing wound sealing clots)

IT 56-87-1, **Lysine**, biological studies
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (histidine replacement of; **mutant** **prourokinase** compns. for lysis of occlusive blood clots while sparing wound-sealing clots)

IT 71-00-1, **Histidine**, biological studies
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (lysine replacement by; **mutant prourokinase** compns. for lysis of occlusive blood clots while sparing wound-sealing clots)

IT 8063-07-8, **Kanamycin**
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
 (**mutant prourokinase** compns. for lysis of occlusive blood clots while sparing wound-sealing clots)

L98 ANSWER 3 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 2004:261901 HCAPLUS
 DN 140:387671
 TI Expression and characterization of recombinant human amino-terminal fragment of **prourokinase**
 AU Wang, Jing; Chen, Xin-Yuan; Sun, Zi-Yong; Yao, Hong-Wei; Chen, Jun-Yong; Liu, Jian-Ning
 CS Institute of Molecular Medicine, Nanjing University, Nanjing, 210093,

Peop. Rep. China

SO Nanjing Daxue Xuebao, Ziran Kexue (2004), 40(1), 66-74
CODEN: NCHPAZ; ISSN: 0469-5097

PB Nanjing Daxue Xuebao Bianjibu

DT Journal

LA Chinese

AB ATF is the amino terminal Ser1-Lys 135 fragment of pro-urokinase (proUK) containing an epidermal growth factor-like (EGF) domain and a kringle domain. It is critically involved in some important functions of proUK. The EGF domain participates in receptor binding and promoting cell adhesion and migration. The kringle domain is associated with the chemotactic action, anti-angiogenic and anti-tumor activities. ATF can bind to the urokinase-type plasminogen activator receptor (uPAR) with high affinity ($K_d = 4 + 10-10$ mol/L) but it is enzymically inactive, and specifically prevent binding of pro-uPA synthesized by tumor cells to the receptors, which inhibits the proliferation and invasion of tumor cells. Fabbrini MS et al. has constructed a chimera consisting of the human ATF fused to a cytotoxin saporin isoform (SAP-3) to target and destroy tumor cells. Therefore, ATF is a potential reagent against cancer. Recent studies suggest that ATF also inhibits the replication, assembly and budding of HIV-1, providing a novel therapeutic strategy for AIDS. In this report, the cDNA fragment encoding ATF was cloned from the endothelial cells of human umbilical vein (HUVEC) by reverse transcriptase polymerase chain reaction (RT-PCR). The ATF gene was inserted between the NdeI-XhoI sites of pET-29a (+) vector to construct recombinant expression plasmid pET-29a(+)/ATF. The host cell strain *E. coli* BL21 (DE3) transformed with pET-29a(+)/ATF was induced with IPTG to overexpress recombinant human ATF (rhATF), as insol. inclusion body. The amount of rhATF expressed accounts for 20% of total bacterial protein. After purification of inclusion body, renaturation, CM cation-exchange chromatog. and Superdex G-75 gel filtration, 15 mg rhATF was obtained from one liter of culture medium with homogeneity greater than 95%. The results of zymograph assay demonstrated that the purified rhATF could block the binding of proUK to the uPAR on U973 cell surface. Kinetic anal. showed that both Glu-plasminogen activation by urokinase and proUK activation by Lys-plasmin were dose-dependently inhibited by rhATF.

IT 82657-92-9P, Prourokinase
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(expression and characterization of recombinant human amino-terminal fragment of prourokinase)

L98 ANSWER 4 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
AN 2004:261890 HCAPLUS
DN 141:64546
TI Novel kringle mutant of prourokinase suppressing tumor growth
AU Cao, Zhong-Wei; Ding, Bi-Sen; Chen, Xin-Yuan; Zhou, Ying-Jiang; Wang, Shi-Quan; Zhang, Jing; Zhu, Zhen-Hua; Chen, Yu-Hong; Liu, Jian-Ning
CS Institute of Molecular Medicine, Nanjing University, Nanjing, 210093, Peop. Rep. China
SO Nanjing Daxue Xuebao, Ziran Kexue (2004), 40(1), 28-33
CODEN: NCHPAZ; ISSN: 0469-5097
PB Nanjing Daxue Xuebao Bianjibu
DT Journal
LA Chinese
AB Kringles of plasminogen and other proteins, obtained by proteolytic fragments, have been reported to display the anti-tumor activity, which represent potent anti-cancer candidates. However, there remains

controversy on whether it is the sequence or the tertiary structure that renders Kringle the anti-tumor activity. In order to address such an issue, we cloned the genes of Kringle of prourokinase and obtained its **mutant** by inserting a previously demonstrated fragment of 16 amino acids from Kringle 5 of plasminogen that manifested anti-tumor activity. The constructed recombinant vectors **DE3** were expressed in **E. coli BL21** (DE3), induced by IPTG. Prourokinase Kringle and the **mutant** were first purified by Ni-NTA affinity chromatog. and then subjected to renaturation. Finally, the folding solns. were applied to CM ion-exchange chromatog. for further purification and concentration. As a result,

appropriately folded proteins with high purity were obtained, which were confirmed by SDS-PAGE anal. To compare the *in vivo* anti-tumor activities of prourokinase Kringle and its **mutant**, male 6-wk C57/BL6 mice were used for tumor study. Lewis lung carcinoma cells were s.c. injected and the anti-tumor efficacy was evaluated on the basis of tumor volume. Here, prourokinase Kringle almost displayed no anti-tumor activity while its **mutant** comparatively stifled the growth of s.c. tumor, illustrating that equipping proteins with certain anti-tumor fragment will inhibit tumor growth and it is the amino acid sequence rather than the tertiary structure of protein that enables several Kringle structures to prevent tumor from growing.

IT 82657-92-9, Prourokinase

RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(novel kringle **mutant** of prourokinase suppressing tumor growth)

L98 ANSWER 5 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:907166 HCAPLUS

DN 138:322

TI Plasma glucosylceramide deficiency as risk factor for thrombosis and modulator of anticoagulant protein C

IN Griffin, John H.; Deguchi, Hiroshi; Fernandez, Jose

PA USA

SO U.S. Pat. Appl. Publ., 32 pp.

CODEN: USXXCO

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2002177563	A1	20021128	US 2002-86943	20020228 <--
	US 6756208	B2	20040629		
	WO 2002102325	A2	20021227	WO 2002-US6340	20020228 <--
	WO 2002102325	A3	20030912		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	EP 1370570	A2	20031217	EP 2002-760992	20020228 <--
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	US 2004132688	A1	20040708	US 2003-739962	20031217 <--
PRAI	US 2001-272103P	P	20010228 <--		
	US 2001-278045P	P	20010322 <--		

US 2002-86943 A3 20020228 <--
 WO 2002-US6340 W 20020228 <--

AB The present invention has determined that exogenously added glucosylceramide (GlcCer) and other neutral glycolipids such as the homologous Glc-containing globotriaosylceramide (Gb3Cer), dose-dependently prolonged clotting times of normal plasma in the presence but not absence of APC:protein S, indicating GlcCer or Gb3Cer can enhance protein C pathway anticoagulant activity. In studies using purified proteins, inactivation of factor Va by APC:protein S was enhanced by GlcCer alone and by GlcCer, globotriaosylceramide, lactosylceramide, and galactosylceramide in multicomponent vesicles containing phosphatidylserine and phosphatidylcholine. Thus, the present invention provides neutral glycolipids such as GlcCer and Gb3Cer, as anticoagulant cofactors that contribute to the antithrombotic activity of the protein C pathway. The present invention has also determined that a deficiency of plasma GlcCer is a risk factor for thrombosis. Methods are provided to determine individuals at risk for thrombosis, methods of treatment as well as methods of screening for antithrombotic factors from neutral glycolipids.

IT 71965-57-6, Globotriaosylceramide
 RL: ANT (Analyte); DGN (Diagnostic use); PAC (Pharmacological activity); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
 (plasma glucosylceramide or other neutral glycolipid deficiency as risk factor for thrombosis and modulator of anticoagulant protein C when given in vesicle form in relation to combination with other agents)

IT 82657-92-9, Prourokinase 82657-92-9D,
 Prourokinase, analogs
 RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
 (plasma glucosylceramide or other neutral glycolipid deficiency as risk factor for thrombosis and modulator of anticoagulant protein C when given in vesicle form in relation to combination with other agents)

RETABLE

Referenced Author (RAU)	Year (R PY)	VOL (R VL)	PG (R PG)	Referenced Work (RWK)	Referenced File
Anon	1998			WO 9856365 A1	HCAPLUS
Anon	2000			WO 0053264 A1	HCAPLUS
Chatterjee, S	1998	18	1523	Arterioscler Thromb	HCAPLUS
Clarke, J	1981	59	412	Can J Biochem.	HCAPLUS
Dawson, G	1976	17	125	J Lipid Res.	HCAPLUS
Debuchi, H	2001	97	1907	Blood	
Deguchi, H	2002	277	8861	J Biol Chem	HCAPLUS
Deguchi, H	2000	97	1743	Proc. Natl Acad Sci	MEDLINE
Fernandez, J	2000	26	115	Blood Cells Mol Dis.	HCAPLUS
Griffin, J	1999	103	219	J. Clin Invest.	HCAPLUS
Hakomori, S	1995	118	1091	J Biochem.	HCAPLUS
Heran, C	2000	389	201	Eu. J. Pharm.	HCAPLUS
Phillips, D	1993	61	344	J. Neurochem.	HCAPLUS
Smirnov, M	1994	269	816	J Biol Chem.	HCAPLUS
Svensson, P	1994	330	517	N Engl J. Med.	MEDLINE

L98 ANSWER 6 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:326876 HCAPLUS

DN 137:273001

TI Prourokinase mutant that induces highly effective clot lysis without interfering with hemostasis

AU Liu, Jian-Ning; Liu, Jian-Xia; Liu, Bei-fang; Sun, Ziyong; Zuo, Jian-Ling; Zhang, Pei-xiang; Zhang, Jing; Chen, Yu-hong; Gurewich, Victor

CS Vascular Research Laboratory, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

SO Circulation Research (2002), 90(7), 757-763

CODEN: CIRUAL; ISSN: 0009-7330

PB Lippincott Williams & Wilkins

DT Journal

LA English

AB **Prourokinase (proUK)** is a zymogenic plasminogen activator that at pharmacol. doses is prone to nonspecific activation to **urokinase**. This has handicapped therapeutic exploitation of its fibrin-specific physiol. properties. To attenuate this susceptibility without compromising specific activation of **proUK** on a fibrin clot, a **Lys 300 → His** mutation (M5) was developed. M5 had a lower intrinsic activity and, therefore, remained stable in plasma at a 4-fold higher concentration than did **proUK**. M5 had a higher 2-chain activity and induced more rapid plasminogen activation and fibrin-specific clot lysis in vitro. Sixteen dogs embolized with radiolabeled clots were infused with saline, **proUK**, tissue plasminogen activator, or M5. The lower intrinsic activity allowed a higher infusion rate with M5, which induced the most rapid and efficient clot lysis (50% clot lysis by ≈600 µg/kg M5 vs. ≈1200 µg/kg **proUK**). In association with this, M5 caused neither a significant increase in the primary bleeding time nor secondary bleeding (total blood loss). By contrast, these measurements increased 4-fold and 5-fold, resp., with **proUK** and >5-fold and 8-fold, resp., with tissue plasminogen activator. Clot lysis by M5 and hemostasis were further evaluated in 6 rhesus monkeys. M5 again induced rapid clot lysis without a significant increase in the primary bleeding time, and secondary bleeding did not occur. In conclusion, a site-directed mutation designed to improve the stability of **proUK** in blood at therapeutic concns. induced superior clot lysis in vitro and in vivo without causing significant interference with hemostasis.

IT 82657-92-9, Prourokinase

RL: PAC (Pharmacological activity); BIOL (Biological study)
(comparison with; **prourokinase mutant** that induces
highly effective clot lysis without interfering with hemostasis)

IT 82657-92-9DP, Prourokinase, histidine 300

mutant, M5

RL: BSU (Biological study, unclassified); PAC (Pharmacological activity);
PRP (Properties); SPN (Synthetic preparation); THU (Therapeutic use); BIOL
(Biological study); PREP (Preparation); USES (Uses)
(**prourokinase mutant** that induces highly effective
clot lysis without interfering with hemostasis)

RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
Carmeliet, P	1994	368	419	Nature	HCAPLUS
Collen, D	1985	72	384	Circulation	MEDLINE
Declerck, P	1991	64	394	Thromb Haemost	
Gurewich, V	1984	73	1731	J Clin Invest	HCAPLUS
Gurewich, V	1988	82	1956	J Clin Invest	HCAPLUS
Harpel, P	1985	260	4432	J Biol Chem	HCAPLUS
Hekman, C	1988	27	2911	Biochemistry	HCAPLUS
Lang, I	1993	87	1990	Circulation	HCAPLUS
Liu, J	1992	31	6311	Biochemistry	HCAPLUS
Liu, J	1996	35	14070	Biochemistry	HCAPLUS
Liu, J	1993	81	980	Blood	HCAPLUS
Liu, J	1992	267	15289	J Biol Chem	HCAPLUS
Liu, J	1995	270	8408	J Biol Chem	HCAPLUS
Liu, J	1991	88	2012	J Clin Invest	HCAPLUS
Meyer, J	1989	1	863	Lancet	
Michels, H	1996	7	766	Blood Coagul Fibrinolysis	HCAPLUS
Michels, R	1995	2	117	J Thromb Thrombolysis	HCAPLUS
Montoney, M	1995	91	1540	Circulation	HCAPLUS
Northeast, A	1995	22	573	J Vasc Surg	MEDLINE

Orini, G	1991	195	691	Eur J Biochem	
Pannell, R	1986	67	1215	Blood	HCAPLUS
Pannell, R	1987	69	22	Blood	HCAPLUS
Pannell, R	1988	81	853	J Clin Invest	HCAPLUS
Sun, Z	1998	37	2935	Biochemistry	HCAPLUS
Sun, Z	1997	272	23818	J Biol Chem	HCAPLUS
Swaim, W	1967	13	1026	Clin Chem	HCAPLUS
Topol, E	1993	329	673	N Engl J Med	
Torr, S	1992	19	1085	J Am Coll Cardiol	HCAPLUS
Van de Werf, F	1984	69	605	Circulation	HCAPLUS
Verde, P	1984	81	4727	Proc Nat Acad Sci	HCAPLUS
Winkler, M	1986	25	4041	Biochemistry	HCAPLUS
Yamamoto, K	1996	97	2440	J Clin Invest	HCAPLUS

L98 ANSWER 7 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:52047 HCAPLUS

DN 136:339530

TI Characterization and large scale preparation of human recombinant prourokinase

AU Chen, Yuhong; Sun, Ziyong; Zhu, Zhenhua; Zhang, Jing; Fu, Yigong; Zhu, Dexu; Liu, Jianning

CS Institute of Molecular Medicine, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Nanjing Daxue Xuebao, Ziran Kexue (2001), 37(4), 407-415

CODEN: NCHPAZ; ISSN: 0469-5097

PB Nanjing Daxue Xuebao Bianjibu

DT Journal

LA Chinese

AB The engineered *E. coli* containing pET29a-prou was cultured in a 10 L seeding tank, and then grew in a 100 L fermentor under IPTG induction. The expressed protein accounted for 20% of the bacteria total proteins. After renaturation, the folding solution was applied to a column of CM-cellulose, and the fraction containing recombinant prourokinase activity further was purified by Superdex 75 gel filtration and depyrogenated by Affiprep polymyxin affinity chromatog. A pilot purification yielded 6 g of purified human recombinant prourokinase from 100 L medium. The purity of the resulting protein was higher than 95 %, and its specific activity was over 120 000 iu/mg. The content of the two chain urokinase was less than 0.5 %, and the trace content of pyrogen, the residual protein and DNA from the host cell all met the requirements for clin. use. The mol. weight of the recombinant prourokinase and the amino acid composition were consistent with the theor. data. The isoelectrophoretic point and peptide mapping of the pro-urokinase were also determined

IT 82657-92-9P, Prourokinase

RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation) (characterization and large scale preparation of human recombinant prourokinase)

L98 ANSWER 8 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:52046 HCAPLUS

DN 137:89065

TI Human prourokinase cDNA gene cloning and engineered strain construction and characterization

AU Chen, Yuhong; Zhang, Jing; Zhu, Zhenhua; Fu, Yigong; Liu, Jianning

CS Institute of Molecular Medicine, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Nanjing Daxue Xuebao, Ziran Kexue (2001), 37(4), 401-406

CODEN: NCHPAZ; ISSN: 0469-5097

PB Nanjing Daxue Xuebao Bianjibu

DT Journal

LA Chinese
 AB The human recombinant prourokinase gene was cloned from the epithelial cells of human umbilical vein by RT-PCR. The pET29a/prouk plasmid was constructed and expressed in *Escherichia coli* BL21(DE3) strain by IPTG induction. The expression product with a 46 kDa was identified by SDS-PAGE, and the protein accounted for 20% of the bacterial total protein as inclusion body. The engineered strain showed the stability of its plasmid maintenance, resuscitation and expression efficiency in storage and regeneration.

IT 82657-92-9, Prourokinase
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (human prourokinase cDNA gene cloning and engineered strain construction and characterization)

L98 ANSWER 9 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 2002:20281 HCPLUS
 DN 136:397679
 TI Characterization of bifunctional chimeric molecule of PRGDWR containing pro-urokinase
 AU Dang, Xin; Yang, Jingxin; Ru, Qiang; Ru, Binggen
 CS National Laboratory of Protein Engineering, College of Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China
 SO Shengwu Huaxue Yu Shengwu Wuli Jinzhan (2001), 28(2), 203-209
 CODEN: SHYCD4; ISSN: 1000-3282
 PB Shengwu Huaxue Yu Shengwu Wuli Jinzhan Bianjibu
 DT Journal
 LA Chinese
 AB The bifunctional chimeric mol. of single-chain urokinase-type plasminogen activator (scu-PA) which inhibits platelet aggregation was studied. The PRGDWR peptide was inserted into the site between Gly118 and Leu119 (called insertion mutant B, InB). The recombinant gene of InB was expressed by *Pichia pastoris*. The secreted protein was purified by metal chelate affinity and strong cation exchange chromatog. The amidolytic ability of mutant InB is 5900 IU/mg, the kinetic consts. is: $K_m, \text{plgInB} = 56.8 \mu\text{mol L}^{-1}$, $k_{\text{cat}}, \text{plgInB} = 0.33 \text{ s}^{-1}$. The kinetic consts. of plasminogen activation reaction is: $K_{\text{InBm}}, \text{plg} = 0.397 \mu\text{mol L}^{-1}$, $k_{\text{InBcat}}, \text{plg} = 0.0164 \text{ s}^{-1}$. Fibrin inhibit the catalytic ability of InB during plasminogen activation, the influence factor is 0.463 (means InB remain 46.3% of the catalytic ability when fibrin was involved in the reaction system). The mutant not only has almost the same catalytic ability as wild type scu-PA, but also has strong ability of anti-platelet aggregation (compared with scu-PA), IC₅₀ of InB is 12.7 $\mu\text{mol L}^{-1}$.

IT 82657-92-9DP, Single-chain urokinase -type plasminogen activator, PRGDWR-containing analogs
 RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); CAT (Catalyst use); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)
 (characterization of bifunctional chimeric mol. of PRGDWR containing pro-urokinase)

L98 ANSWER 10 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 2001:461655 HCPLUS
 DN 136:194764
 TI Application of gel chromatography renaturing way on low molecular single-chain urokinase mutant (DscuPA-32K)
 AU Jiao, Jianwei; Yu, Meimin; Ru, Binggen
 CS National Laboratory of Protein Engineering, College of Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China
 SO Shengwu Gongcheng Xuebao (2001), 17(3), 300-303

CODEN: SGXUED; ISSN: 1000-3061
 PB Kexue Chubanshe
 DT Journal
 LA Chinese
 AB The application of gel chromatog. renaturing method on low mol. single-chain urokinase mutant (DscuPA-32K) was studied. A recombinant mutant gene with thrombolytic and antithrombolytic bifunction was expressed in *E. coli*. Owing to two reasons of high mol. weight and over expression, dscuPA existed in inclusion body form. The protein of inclusion body was inactive protein. To obtain active protein, inclusion bodies should be denatured and then renatured. A novel, named gel-chromatog. column renaturation method was performed. Compared with traditional renaturation method, this refolding approach had obvious advantages including low cost and high recovery.

IT 82657-92-9, Single-chain urokinase
 RL: PEP (Physical, engineering or chemical process); PROC (Process) (application of gel chromatog. renaturing method on low mol. single-chain urokinase mutant (DscuPA-32K))

L98 ANSWER 11 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 2001:350846 HCPLUS
 DN 135:16011
 TI Catalysis-related function of kringle domain of single chain urokinase-type plasminogen activator
 AU Dang, Xin; Ji, Jian-guo; Ru, Qiang; Yang, Jing-xin; Yu, Mei-min; Ru, Bing-gen
 CS National Lab. of Protein Engineering, Peking University, Beijing, 100871, Peop. Rep. China
 SO Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (2001), 17(2), 219-225
 CODEN: ZSHXF2; ISSN: 1007-7626
 PB Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
 DT Journal
 LA Chinese
 AB Kringle domain is a conservative domain that exists in many thrombus forming and thrombolysis related factors. The primary structure of Kringle domain is high conserved (compared with other domains in those factors). To study the function of Kringle domain of single chain urokinase-type plasminogen activator (scu-PA), an Kringle domain inserted mutant of scu-PA was constructed by inserting PRGDWR peptide at the site between Gly118 and Leu119 (Insert mutant B, InB) and the kinetic consts. of thrombolytic related reactions were detected. Km value of hydrolytic reaction of S-2444 catalyzed by the two mol. was not significantly different (60.4 and 56.8 $\mu\text{mol}\cdot\text{L}^{-1}$ resp.), but the Km value of InB (0.33 s^{-1}) was decreased a lot compared with the kcat of scu-PA (7.31 s^{-1}). The Km value of InB (0.397 $\mu\text{mol}\cdot\text{L}^{-1}$) of the activation reaction of plasminogen was 40% lower than the Km value of scu-PA (0.648 $\mu\text{mol}\cdot\text{L}^{-1}$), but the kcat value of InB (0.0165 s^{-1}) was over 70% lower than that of scu-PA (0.0626 s^{-1}). These results suggest that Kringle domain of scu-PA is related with the catalytic activity of scu-PA, but has no influence on the affinity between scu-PA and its substrate.

IT 82657-92-9, Single chain urokinase -type plasminogen activator
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study) (Kringle domain of single chain urokinase -type plasminogen activator (scu-PA) is related with the catalytic activity of scu-PA, but has no

influence on affinity between **scu-PA** and its substrate)

L98 ANSWER 12 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 2001:191009 HCAPLUS
 DN 135:339965
 TI Thioredoxin reductase-deficient **E. coli** mutant enhances expression into solution of recombinant proteins containing Cys residues
 AU Tong, Qin; Yang, Yungui; Zhang, Huitang; Chen, Yan; Yang, Shengli; Gong, Yi
 CS Shanghai Research Center of Biotechnology, Chinese Academy of Sciences, Shanghai, 200233, Peop. Rep. China
 SO Shengwu Huaxue Yu Shengwu Wuli Xuebao (2001), 33(1), 30-34
 CODEN: SHWPAU; ISSN: 0582-9879
 PB Shanghai Kexue Jishu Chubanshe
 DT Journal
 LA Chinese
 AB A 3D artificial protein, a salmon calcitonin hexopolymer, a salmon calcitonin octopolymer, and a human prourokinase, was expressed in cytoplasm of **E. coli** GJ980 (trxB-) mutant. The recombinant proteins contained cysteine residues of different length of 12-22 residues. The mutation was mapped to the gene for thioredoxin reductase and may eliminate the activity of enzyme, and the effect was related to the sulfhydryl reducing potential of cytoplasm. Recombinant salmon calcitonin hexopolymer, salmon calcitonin octopolymer, and human prourokinase had more soluble form in cytoplasm of GJ980 mutants than in wild-type strain, but 3D-protein with no cysteine residue remained in insol. form. The results showed that the formation of disulfide bonds in cell cytoplasm GJ980 (trxB-) strain may play an important role in form correct folding and soluble expression of the recombinant proteins.

IT 82657-92-9P, ProUrokinase
 RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
 (human; thioredoxin reductase-deficient **E. coli** mutant enhances expression into solution of recombinant proteins containing Cys residues)

L98 ANSWER 13 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 2001:124859 HCAPLUS
 DN 135:252488
 TI Studies on expression of human pro-urokinase in **Escherichia coli**
 AU Wang, Tao; Zhou, Xianwan; Hu, Meihao
 CS College Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China
 SO Beijing Daxue Xuebao, Ziran Kexueban (2000), 36(6), 802-807
 CODEN: PCTHAP; ISSN: 0479-8023
 PB Beijing Daxue Chubanshe
 DT Journal
 LA Chinese
 AB Studies were made on the enhancement of expression of human pro-Urokinase(Pro-UK) cDNA in **Escherichia coli**. By means of PCR, the signal peptide DNA sequence was deleted. For study of the limited factors in expression of pro-Urokinase, the pro-UK gene was divided into three fragments, and they were expressed in **E. coli** BL21(DE3). The expression of middle fragment is very low, because there are several rare codon AGG (Arg) in it. Using a new bacteria **E. coli** BL21-Codon Plus-RIL to introduce DNA Y gene coding tRNA^{agg/aga} (Arg) to recognize the rare

codon AGG, the expression level of the middle fragment was enhanced to 10%-20% of total cell protein and the expression of the intact pro-UK was enhanced to 5% by more than 10 folds.

IT 82657-92-9P, Pro-Urokinase

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(expression of human pro-urokinase in Escherichia coli)

L98 ANSWER 14 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 2000:664901 HCPLUS

DN 134:1977

TI A region in domain II of the urokinase receptor required for urokinase binding

AU Bdeir, Khalil; Kuo, Alice; Mazar, Andrew; Sachais, Bruce S.; Xiao, Weizhong; Gawlak, Susan; Harris, Scott; Higazi, Abd Al-Roof; Cines, Douglas B.

CS Departments of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA

SO Journal of Biological Chemistry (2000), 275(37), 28532-28538
CODEN: JBCHA3; ISSN: 0021-9258

PB American Society for Biochemistry and Molecular Biology

DT Journal

LA English

AB The urokinase receptor is composed of three homologous domains based on disulfide spacing. The contribution of each domain to the binding and activation of single chain urokinase (scuPA) remains poorly understood. In the present paper we examined the role of domain II (DII) in these processes. Repositioning DII to the amino or carboxyl terminus of the mol. abolished binding of scuPA as did deleting the domain entirely. By using alanine-scanning mutagenesis, we identified a 9-amino acid continuous sequence in DII (Arg137-Arg145) required for both activities. Competition-inhibition and surface plasmon resonance studies demonstrated that mutation of Lys139 and His143 to alanine in soluble receptor (suPAR) reduced the affinity for scuPA .apprx.5-fold due to an increase in the "off rate". Mutation of Arg137, Arg142, and Arg145, each to alanine, leads to an .apprx.100-fold decrease in affinity attributable to a 10-fold decrease in the apparent "on rate" and a 6-fold increase in off rate. These differences were confirmed on cells expressing variant urokinase receptor. SuPARK139A/H143A displayed a 50% reduction in scuPA-mediated plasminogen activation activity, whereas the 3-arginine variant was unable to stimulate scuPA activity at all. Mutation of the three arginines did not affect binding of a decamer peptide antagonist of scuPA known to interact with DI and DIII. However, this mutation abolished both the binding of soluble DI to DII-III in the presence of scuPA and the synergistic activation of scuPA mediated by DI and wild type DII-DIII. These data show that DII is required for high affinity binding of scuPA and its activation. DII does not serve merely as a spacer function but appears to be required for interdomain cooperativity.

IT 71-00-1, L-Histidine, biological studies

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(His143; 9-amino acid sequence in domain II of urokinase receptor (Arg137-Arg145) required for urokinase binding)

IT 56-87-1, L-Lysine, biological studies

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Lys139; 9-amino acid sequence in domain II of urokinase receptor (Arg137-Arg145) required for urokinase binding)

IT 82657-92-9, Single chain urokinase

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

(region in domain II of urokinase receptor required for urokinase binding)

L98 ANSWER 15 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 2000:363770 HCPLUS
 DN 133:291675
 TI Construction of pro-urokinase mutant
 Glu151-Glu154-mscu-PA and characterization of its kinetic properties
 AU Liu, Wei; Zhu, Hui; Shi, Wei; Ma, Zhong
 CS Department of Biochemistry and State Laboratory of Pharmaceutical Biotechnology, Nanjiang University, Nanjiang, 210093, Peop. Rep. China
 SO Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (2000), 16(2), 188-193
 CODEN: ZSHXF2; ISSN: 1007-7626
 PB Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
 DT Journal
 LA Chinese
 AB Rscu-PA and its mutant constructed in vitro by site-directed mutagenesis of Lys151, Arg154 in rscu-PA to Glu151, Glu154(mscu-PA) were both expressed in *Escherichia coli*. After in vitro denaturation and renaturation, the rscu-PA and mscu-PA were purified to homogeneity by Zn²⁺ selective precipitation, anti-u-PA IgG-Sepharose CL 4B affinity chromatog. The activation by plasmin of mscu-PA was 40% lower than that of rscu-PA. Mscu-PA and rscu-PA were found essentially identical in Glu-plasminogen activation. After activation by plasmin, the kinetic consts. for the resultant mtcu-PA against synthetic substrate S2444 hydrolysis were found about 90% of rtcu-PA. Although the Km of two-chain mtcu-PA against Glu-plasminogen was similar to that of rtcu-PA, mtcu-PA had a lower enzymic activity (about 80% that of rtcu-PA) due to a reduction of Kcat. In caseinolytic system, with the fibrin and plasminogen, mscu-PA could speed up the decomposition of casein more than rscu-PA, which suggested that mtcu-PA had some fibrin-specificity.

L98 ANSWER 16 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 2000:344507 HCPLUS
 DN 133:291639
 TI High level expression of a mutant (K151E, R154G) of single chain urokinase-type plasminogen activator in silkworm
 AU Peng, Rui; Yu, Zhe-yong; Zang, Yu-hui; Qin, Jun-chuan
 CS Department of Biochemistry and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China
 SO Nanjing Daxue Xuebao, Ziran Kexue (2000), 36(2), 235-238
 CODEN: NCHPAZ; ISSN: 0469-5097
 PB Nanjing Daxue
 DT Journal
 LA English
 AB The cDNA of a mutant of single chain urokinase-type plasminogen activator (rscu-PA) gene in silkworm, mscu-PA (K151E, R154G), was reconstructed to include the natural urokinase (u-PA or UK) signal peptide sequences, which was obtained by the PCR method. In order to introduce the whole mscu-PA gene (including the signal peptide sequence) into BmNPV genome and put it under the control of the polyhedrin (ph) promoter, a BmNPV-derived transfer vector pBE284 was used as well as pVL1392, a transfer vector derived from AcNPV. Based on the sequence homol. of the ph promoter and its flanking region in BmNPV and AcNPV genome, the 5' non-encoding sequence (including promoter) of the ph gene from pVL1392 replaced that in wild type BmNPV DNA via in vivo homologous recombination. The two polyhedrin-recombinant viruses were isolated and identified by dot hybridization using a labeled scu-PA cDNA probe, and were designated BmNPV-mscu-PA-B (by pBE284) and BmNPV-mscu-PA-V (by pVL1392), resp. The protein mscu-PA was secreted into both the

culture medium and hemolymph of the larvae, and exhibited high biol. activity. The highest yield of the recombinant protein was about 150 µg/mL. Furthermore, compared with those infected with BmNPV-mscu-PA-B, the yield of mscu-PA was increased threefold in cultured BmN cells and 2.3 times in silkworm larvae infected with BmNPV-mscu-PA-V. The highest expression may be due to the structure of pVL1392. In pVL1392, the entire 5'-untranslated region of (UTR) ph gene is present as well as the first 35 nucleotides of the ph coding sequences, except that ATG has been modified to ATT to prevent the synthesis of a fusion protein. This may play an important role in the translation of the recombinant protein in insect cells. It may increase the RNA stability and translational efficiency by resembling the 5' portion of the natural ph mRNA or by forming a longer 5' UTR. The transfer vector pVL1392 and the silkworm expression system could be used as one of the most economical ways of overproducing mscu-PA protein.

IT 82657-92-9P, Single chain urokinase
-type plasminogen activator
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP
(Preparation)
(high level expression of a mutant (K151E, R154G) of
single chain urokinase-type plasminogen
activator in silkworm)

RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
Jarvis, D	1990	42	181	J Cell Biochem	HCAPLUS
Meada, S	1989	I	167	Invertebrate Cell Sy	
O'Reilly, D	1992			A Laboratory Manual	
Peng, G	1997	42	972	Chinese Science Bull	

L98 ANSWER 17 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2000:344480 HCAPLUS

DN 133:131555

TI Construction and characterization of two mutants of pro-urokinase (Ala175 →Ser, Tyr18.fwdarw .His and Ala175→Ser, Tyr187→ His)

AU Shi, Wei; Zhu, Hui; Xue, Yu-ming; Tang, Tang; Ma, Zhong

CS Department of Biochemistry, National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Nanjing Daxue Xuebao, Ziran Kexue (2000), 36(2), 208-212

CODEN: NCHPAZ; ISSN: 0469-5097

PB Nanjing Daxue

DT Journal

LA Chinese

AB Human single chain urokinase-type plasminogen activator (scu-PA, also named pro-UK) is an important thrombolytic agent in therapy of thrombosis. The activation of plasminogen on the surface of fibrin induced by pro-UK, is much specific and effective, resulting very small tendency of bleeding from system lytic state. Therefore, great importance has been attached to scu-PA in clinics. Though pro-UK has some selectivity for fibrin, its higher selectivity for fibrin in human bodies was counteracted by its higher intrinsic activity when it was used in large doses. The risk of bleeding still remains. It was discovered that the stretch of 297.appx.313 amino acids in scu-PA formed a loop.

Lys300, the only pos. charge amino acid in the loop might interact with Asp355 which was sited near the active center by charge attraction. Thus Ser365 was pulled, Ser365 Asp225, His204 might form the active center on the 3-D structure resulted in the high intrinsic activity of pro-UK. Besides, according to the data of crystal structure of chymotrypsin, Asp194, His40 and Ser32 formed a zymogen triad,

which keeps the inactive conformation of chymotrypsin. As a member of the zymogens of serine protease family, pro-UK lacks the zymogen triad, His40 and Ser32 were replaced resp. by Tyr187 and Ala175. This was also the structure basis of pro-UK's high enzymic activity. To reduce the intrinsic activity of pro-UK, 2 mutant genes of pro-urokinase, muk1 (Ala175→Ser, Tyr187.fwdarw .His, Lys300 .fwdarw.His) and muk2 (Ala175→Ser, Tyr187→His) were constructed by site directed mutagenesis, and were expressed in *E. Coli* BL21. The expressed inclusion body was treated by denaturation and renaturation, and purified by SP-sepharose ion-exchange chromatog. and Benzamidine Sepharose affinity adsorption. Using the synthetic substrate S2444, the intrinsic activity and the enzymic activity of two-chain form of muk1 and muk2 were measured. The intrinsic activities of muk1 and muk2 were 8-fold and 2.5-fold lower than that of pro-urokinase, resp. The enzymic activity of two-chain muk1 was 1.5-fold higher than that of urokinase and the activity of muk2 was the same as wild type urokinase. The mechanism and the structure basis of a much higher intrinsic catalytic activity than other zymogens of the serine protease family was discussed.

IT 82657-92-9P, Single-chain urokinase -type plasminogen activator
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); PROC (Process)
 (construction and characterization of two mutants (muk1 and muk2) of pro-urokinase (Ala175 →Ser, Tyr187.fwdarw.His and Ala175→Ser, Tyr187→His))

L98 ANSWER 18 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 2000:141655 HCAPLUS
 DN 133:39761
 TI Construction and characterization of a mutant of single -chain urokinase-type plasminogen activator (Ser175-His187-mscu-PA)
 AU Xue, Yu-Ming; Zhu, Hui; Shi, Wei; Liu, Wei; Liu, Jian-Ning; Ma, Zhong
 CS Department of Biochemistry, National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China
 SO Shengwu Huaxue Yu Shengwu Wuli Xuebao (2000), 32(1), 26-30
 CODEN: SHWPAU; ISSN: 0582-9879
 PB Shanghai Kexue Jishu Chubanshe
 DT Journal
 LA Chinese
 AB Single-chain urokinase-type plasminogen activator (scu-PA) is the precursor of double-chain urokinase (tcu-PA), which has a much higher intrinsic catalytic activity than other zymogens of the serine protease family. To restore the "zymogen triad" of Asp-His-Ser in the serine protease family, the mutant gene of scu-PA (mscu-PA, Ala175 → Ser175, Tyr187 → His187) was constructed by the method of oligonucleotide-directed, site-specific mutagenesis in order to reduce its intrinsic catalytic activity. mscu-PA was expressed in *E. coli* BL21. After denaturation and renaturation in vitro, the mscu-PA was purified to homogeneity by SP-Sepharose ion-exchange chromatog., Sephadryl S-200 chromatog. and Benzamidine-Sepharose affinity adsorption, and the mutant mscu-PA had the same activity to plasmin as scu-PA. The catalytic efficiency (measured by kcat/Km) of the mutant to synthetic substrate S2444 was 2.5-fold lower than that of scu-PA, and the activity against Glu-plasminogen was also reduced.

After activation by plasmin, mtcu-PA and tcu-PA had similar catalytic efficiency against S2444 and Glu-plasminogen. The intrinsic catalytic activity of mscu-PA may be reduced after restoring the "zymogen triad".

L98 ANSWER 19 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 2000:55660 HCAPLUS
 DN 132:203955
 TI The expression of **proUK** in *Escherichia coli*:
 the vgb promoter replaces IPTG and coexpression of argU compensates for rare codons in a hypoxic induction model
 AU Jiang, Lan; Yang, Yonghua; Chatterjee, Shampa; Seidel, Bertolt; Wolf, Gerald; Yang, Shengli
 CS Shanghai Research Center of Biotechnology, Chinese Academy of Sciences, Shanghai, 200233, Peop. Rep. China
 SO Bioscience, Biotechnology, and Biochemistry (1999), 63 (12), 2097-2101
 CODEN: BBBIEJ; ISSN: 0916-8451
 PB Japan Society for Bioscience, Biotechnology, and Agrochemistry
 DT Journal
 LA English
 AB The expression of the **proUK** gene was improved by the coexpression of the argU gene cloned in a moderate copy number vector. As the **proUK** gene contains 2% AGG/AGA codons, which is much higher than the normal frequency in *E. coli*, about 0.14-0.21%, the argU gene cloned in a multicopy plasmid was coexpressed with the **proUK** expression vector in these expts. In *E. coli* strain **BL21(DE3)**, IPTG is known to induce the expression of T7 RNA polymerase gene and this enzyme can transcribe the **proUK** gene under the control of the T7 promoter leading to expression of **proUK**. To replace IPTG by a cheaper alternative on a large scale, a plasmid was constructed in which the vgb promoter - which is known to be activated by the onset of hypoxic conditions - controls T7 RNA polymerase gene expression. Low oxygen conditions were then used to activate the vgb promoter causing T7 RNA polymerase gene expression and finally leading to the expression of **proUK** as inactive inclusion bodies. These expts. on a large scale in a bioreactor show that the expression of **proUK** accounts for .apprx.30% of total protein after about 6 h of anaerobic cultivation, so the presented model represents an economical alternative to IPTG induction.

IT 82657-92-9P, Pro-urokinase

RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)

(expression of **proUK** in *Escherichia coli*

so that the vgb promoter replaces IPTG and coexpression of argU compensates for rare codons in a hypoxic induction model)

RETABLE

Referenced Author (RAU)	Year (R PY)	VOL (R VL)	PG (R PG)	Referenced Work (RWK)	Referenced File
Brinkmann, U	1989	85	109	Gene	HCAPLUS
Bruke, S	1995	77	1025	Thromb Haemost	
Calderone, T	1996	262	407	J Mol Biol	HCAPLUS
Chen, G	1994	8	2641	Genes Dev	HCAPLUS
Credo, R	1995	6	8S	J Vasc Interv Radicul	MEDLINE
Di Minno, G	1989	21	153	Pharmacol Res	MEDLINE
Dikshit, K	1988	70	377	Gene	HCAPLUS
Dikshit, K	1989	135	2601	J Gen Microbiol	HCAPLUS
Dikshit, K	1989	135	2601	J Gen Microbiol	HCAPLUS
Dikshit, K	1990	18	4149	Nucleic Acids Res	HCAPLUS
Hua, Z	1996	220	131	Biochem Biophys Res	HCAPLUS
Joshi, M	1994	202	535	Biochem Biophys Res C	HCAPLUS
Li, F	1995	7	113	Chin J Biotechnol	

Saxena, P	1992	174	1956	J Bacteriol	HCAPLUS
Spanjaard, R	1990	18	5031	Nucleic Acids Res	HCAPLUS
Spanjaard, R	1988	85	7967	Proc Natl Acad Sci	HCAPLUS
Spiecker, M	1994	19	326	Herz	MEDLINE
Vieira, J	1991	100	189	Gene	HCAPLUS
Wang, R	1991	100	195	Gene	HCAPLUS

L98 ANSWER 20 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1999:816585 HCAPLUS

DN 132:289346

TI Construction and expression of a recombinant antibody-targeted plasminogen activator

AU Yang, Jiashu; Jiang, Pengchen; Ru, Binggen

CS National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China

SO Beijing Daxue Xuebao, Ziran Kexueban (1999), 35(4), 487-495
CODEN: PCTHAP; ISSN: 0479-8023

PB Beijing Daxue Chubanshe

DT Journal

LA Chinese

AB A novel plasminogen activator was constructed using scFv SZ51 as targeted mol., and scu-PA-32k as effect mol. SZ51 was a monoclonal antibody of GMP140 on activated human platelets. Polymerase chain reaction (PCR) was used to amplify the region of VK and VH from Fab of SZ51, and scu-PA-32k(leu144-leu411) from urokinase gene, resp. These fragments were joined together and inserted into the expression vector, pET-5a, via a NdeI site. After transforming into *E. coli* BL21 (DE3) and inducing with IPTG, the recombinant protein was expressed in inclusion bodies. Western-Blotting showed that the protein could interact weakly with the multiple clonal antibody of urokinase in 8 M Urea. After renaturation and partial purification, the product had a strong fibrinolytic activity through activating plasminogen on fibrin plate, the specific activity was about 17,500 IU/mg, which showed the recombinant protein retained the activity of u-PA. The yield was almost 1.5 mg/100g wet bacteria.

L98 ANSWER 21 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1999:587341 HCAPLUS

DN 132:147326

TI Oxygen-regulated expression of heterologous gene in *Escherichia coli*

AU Tong, Qin; Yang, Shengli; Gong, Yi

CS Shanghai Research Center of Biotechnology, The Chinese Academy of Sciences, Shanghai, 200233, Peop. Rep. China

SO Shengwu Gongcheng Xuebao (1999), 15(3), 322-326
CODEN: SGXUED; ISSN: 1000-3061

PB Kexue Chubanshe

DT Journal

LA Chinese

AB The expression of *Vitreosilla* Hb gene (vgb) is regulated by the dissolved oxygen consistence in *E. coli*. A new system for expressing heterologous gene in *E. coli* regulated by dissolved oxygen consistence was constructed. It includes a host bacteria GJ100, which contains T7 RNA polymerase gene controlled by vgb promoter, and an expression vector on which the heterologous gene is under the control of T7 promoter. The results indicated that *E. coli* thioredoxin A, IgG binding domain of *Staphylococcus* protein A (ZZ), snake neurotoxin, salmon calcitonin hexa-polymer, human interleukin II (IL2) and human pro-urokinase genes could be expressed efficiently. The expression level of all genes is more than 30% of total cellular protein.

IT 82657-92-9P, Prourokinase
 RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP
 (Preparation)
 (heterologous expression of; oxygen-regulated expression of
 heterologous gene in *Escherichia coli*)

L98 ANSWER 22 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1999:575396 HCPLUS
 DN 131:282177
 TI Construction and expression of a novel **chimeric** protein
 consisting the A chain of tissue-type plasminogen activator and the
 B chain of **pro-urokinase**
 AU Zhao, Chunmei; Zhang, Hongtao; Hu, Meihao
 CS National Laboratory of Protein Engineering and Plant Genetic Engineering,
 Peking University, Beijing, 100871, Peop. Rep. China
 SO Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (1999), 15(4),
 528-531
 CODEN: ZSHXF2; ISSN: 1007-7626
 PB Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
 DT Journal
 LA Chinese
 AB A hybrid cDNA tu-pa, which contained Ser1-Thr263 of tissue-type
 plasminogen activator (t-PA) and Ser138-Leu411 of **pro-**
urokinase (pro-UK) was constructed via
 recombinant DNA technol. and site-directed mutagenesis.
 And it was expressed in the baculovirus system. The activity on fibrin
 plate of the cell culture was 500 IU/mL. The cell culture was purified by
 immunoaffinity chromatog. SDS-PAGE and Western-blot showed that the mol.
 weight of tu-PA was about 60,000. The specific activity of tu-PA was 200,000
 IU/mg protein. The fibrin affinity specificity of tu-PA was much higher
 than that of **pro-UK**.

IT 82657-92-9P, Prourokinase
 RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP
 (Preparation)
 (expression of a novel **chimeric** protein consisting of A chain
 of tissue-type plasminogen activator and B chain of
pro-urokinase)

L98 ANSWER 23 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1999:185499 HCPLUS
 DN 131:14629
 TI High level expression of the deleted and **mutated**
 urokinase-single chain antibody Fv fusion gene in
Escherichia coli
 AU Wang, Xiang; Yu, Weiyuan
 CS Institute of Biotechnology, Academy of Military Medical Sciences, Beijing,
 100071, Peop. Rep. China
 SO Shengwu Gongcheng Xuebao (1999), 15(1), 23-27
 CODEN: SGXUED; ISSN: 1000-3061
 PB Kexue Chubanshe
 DT Journal
 LA Chinese
 AB The fusion gene of a specific anti-human fibrinogen D-dimer single chain
 antibody (scFv) and low mol. weight **single chain**
urokinase (scu-PA-32K) was restricted, spliced
 and digested by exonuclease Bal31 to obtain a series of deletion
 mutants, and their expression in *E. coli*
 revealed that the key sequence which reduced its expression level resides
 in the range from 841 bp to 851 bp, in which tandem AGG codons (encoding
 arginine, rarely used in *E. coli*) exist. By PCR
 mediated site-mutation, we altered two AGG codons to CGT codons,
 and the **mutant** was more efficiently translated in *E.*
coli; the expression level turned out to be about 30% of the total

bacterial proteins while that of the non-mutated nature gene was 2-3%.

L98 ANSWER 24 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1998:757111 HCAPLUS
 DN 130:105814
 TI Fusion expression of human pro-urokinase with
E. coli thioredoxin
 AU Sun, Ai-Long; Hua, Zi-Chun; Yao, Jun; Yang, Yong-Hua; Yin, Da-Qiang
 CS Pharmaceutical Biotechnology Key Laboratory, Department of Biochemistry,
 Nanjing University, Nanjing, 210093, Peop. Rep. China
 SO Biochemistry and Molecular Biology International (1998), 46(3),
 479-486
 CODEN: BMBIES; ISSN: 1039-9712
 PB Academic Press
 DT Journal
 LA English
 AB Human pro-urokinase (pro-UK) was cloned into plasmid pET32b and fused to the *E. coli* thioredoxin (trxA). When expressed in *E. coli* AD494(DE3), the fusion protein Trx-pro-UK accumulated as insol. inclusion bodies and amounted to 35% of total cellular proteins. When co-expressed with mol. chaperones human protein disulfide isomerase (PDI) and *E. coli* GroESL, all the expressed products still existed in the form of insol. inclusion bodies. (c) 1998 Academic Press.
 IT 82657-92-9P, Pro-urokinase
 RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)
 (human; fusion expression of human pro-urokinase with *E. coli* thioredoxin)

RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
Cai, H	1994	269	24550	J Biol Chem	HCAPLUS
Caspers, P	1994	40	635	Cell Mol Biol	HCAPLUS
Collen, D	1980	43	77	Thromb Haemost	HCAPLUS
Dale, G	1994	7	925	Protein Eng	HCAPLUS
Derman, A	1993	262	1744	Science	HCAPLUS
Golobinoff, P	1989	337	44	Nature	
Hayer-Hartl, M	1995	269	836	Science	HCAPLUS
Hibino, Y	1988	52	329	Agric Biol Chem	HCAPLUS
Hiramatsu, R	1991	99	235	Gene	HCAPLUS
Holmes, W	1985	3	923	Bio/Technology	HCAPLUS
Hua, Z	1997	220	131	Biochem Biophys Res	
Hua, Z	1994	33	1215	Biochem Mol Biol Int	HCAPLUS
Hua, Z	1996	39	1093	Biochem Mol Biol Int	HCAPLUS
Humphreys, D	1995	270	28210	J Biol Chem	HCAPLUS
LaVallie, E		11	187	Bio/Technology	HCAPLUS
Laemmli, U	1970	227	680	Nature	HCAPLUS
Laminet, A	1990	9	2315	EMBO J	HCAPLUS
Maniatis, T	1989			Molecular Cloning: A	
Melnick, L	1990	265	801	J Biol Chem	HCAPLUS
Mizobata, T	1992	267	17773	J Biol Chem	HCAPLUS
Nelles, L	1987	262	5682	J Biol Chem	HCAPLUS
Orsini, G	1991	195	691	Eur J Biochem	HCAPLUS
Ostermeier, M	1996	271	10616	J Biol Chem	HCAPLUS
Ploug, J	1957	24	278	Biochim Biophys Acta	HCAPLUS
Puig, A	1994	269	7764	J Biol Chem	HCAPLUS
Roman, L	1995	92	8428	Proc Natl Acad Sci U	HCAPLUS
Surek, B	1991	32	388	Applied Microbiology	

Winkler, M	1985	3	990	Bio/Technology	HCAPLUS
Winkler, M	1985	25	4041	Biochemistry	HCAPLUS
Wun, T	1982	257	7262	J Biol Chem	HCAPLUS

L98 ANSWER 25 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1998:631425 HCAPLUS

DN 129:240872

TI **Escherichia coli** protease triple deletion mutant as host cell for stable expression of normally unstable proteins

IN Kanemori, Masaaki; Yanagi, Hideki; Yura, Takashi

PA HSP Research Institute, Inc., Japan

SO Eur. Pat. Appl., 14 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 866132	A2	19980923	EP 1998-104907	19980318 <--
	EP 866132	A3	19991208		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 10313863	A2	19981202	JP 1998-31718	19980213 <--
	JP 3325512	B2	20020917		
	CA 2226399	AA	19980919	CA 1998-2226399	19980316 <--
PRAI	JP 1997-85914	A	19970319 <--		

AB The **Escherichia coli** mutant carrying a triple deletion mutation in the *hslV/U* gene, the *clpPX* gene, and *lon* gene, and possessing a function to stabilize an unstable protein expressed in **Escherichia coli**, a method for preparing the **Escherichia coli** mutant, a method for stably expressing an unstable protein in **Escherichia coli** by using the **Escherichia coli** mutant, a method for stabilizing an unstable introducing an expression vector carrying a gene encoding a foreign protein into the **Escherichia coli** mutant, and a method for preparing a foreign protein using the transformant. Thus, the **E. coli** mutant strain containing the triple deletion mutation is useful for stable expression of normally unstable proteins such as σ 32, a pollen antigen Cryj2, and human prourokinase.

IT 82657-92-9, Prourokinase

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(stable expression of; **Escherichia coli** protease triple deletion mutant as host cell for stable expression of normally unstable proteins)

L98 ANSWER 26 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1998:415434 HCAPLUS

DN 129:226262

TI High-level expression in **Escherichia coli** and purification of human pro-urokinase cDNA

AU Peng, Guihong; Ma, Zhong; Xue, Yuming; Chen, Yuhong; Zhu, Dexu

CS Department of Biochemistry, National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Shengwu Gongcheng Xuebao (1997), 13(4), 362-367

CODEN: SGXUED; ISSN: 1000-3061

PB Kexue Chubanshe

DT Journal

LA Chinese

AB A chemical synthesized human pro-urokinase (pro-UK) cDNA was cloned into the expression vector pET-11d, and

expressed in **E. coli** BL21(DE3)

pLySS under the control of T7 promoter. The expression level of the recombinant pro-UK was over 15% of total bacterial proteins as inclusion bodies. The specific activity of the purified human pro-UK was about 110000 IU/mg.

IT 82657-92-9P, Pro-urokinase

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(expression in **Escherichia coli** and purification of human pro-urokinase cDNA)

L98 ANSWER 27 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1998:261401 HCPLUS

DN 129:92056

TI Construction of **urokinase mutant** Glu154-**mtcu-PA** and characterization of its properties

AU Peng, Guihong; Ma, Zhong; Xue, Yuming; Chen, Yuhong; Zhu, Dexu

CS Department of Biochemistry and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Shengwu Huaxue Yu Shengwu Wuli Xuebao (1997), 29(6), 547-552

CODEN: SHWPAU; ISSN: 0582-9879

PB Shanghai Kexue Jishu Chubanshe

DT Journal

LA Chinese

AB The recombinant single chain

urokinase-type plasminogen activator (rscu-PA) and a mutant constructed by in vitro site- specific **mutagenesis** of Arg154 in rscu-PA to Glu154 (Glu154-mscu- PA) were both expressed in **E.coli**. The expressed products were both purified to homogeneity by in vitro denaturation and renaturation with Zn²⁺ selective precipitation and immuno-affinity chromatog. The plasmin sensitivity assay indicated that the activation of this single chain Glu154-mscu-PA by plasmin was essentially identical to that of rscu-PA. After activation by plasmin, the kinetic consts. against synthetic substrate S2444 of the resulted 2 chain form of Glu154- mschu-PA (Glu154-**mtcu-PA**) and that of rscu-PA (rtcu-PA) were 87 and 80 μ M, resp. It indicate that the catalytic active site of the Glu154- mtcu-PA is not changed by the mutation. Yet both 125I-fibrin plasma-clot lysis and fibrinogenolysis in plasma showed that the Glu154-**mtcu-PA** possessed a better affinity and selectivity for fibrin than rtcu-PA or even better than rscu-PA.

L98 ANSWER 28 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1997:637172 HCPLUS

DN 127:328291

TI Identification of a **flexible loop** region (297-313) of **urokinase-type plasminogen activator**, which helps determine its catalytic activity

AU Sun, Ziyong; Jiang, Yongping; Ma, Zong; Wu, Hui; Liu, Bei-Fang; Xu, Yuming; Tang, Wei; Cheno, Yuhong; Li, Cuizhen; Zhu, Dexu; Gurewich, Victor; Liu, Jian-Ning

CS Vascular Research Laboratory, Institute for Prevention of Cardiovascular Disease, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02215, USA

SO Journal of Biological Chemistry (1997), 272(38), 23818-23823
CODEN: JBCHA3; ISSN: 0021-9258

PB American Society for Biochemistry and Molecular Biology

DT Journal

LA English

AB Prourokinase has much higher intrinsic catalytic activity than other zymogens of the serine protease family. Lys300(c143) ("cnnc" next to the position nos. of residues indicates chymotrypsin numbering) in an apparent "flexible loop" region

(297-313) was previously shown to be an important determinant of this intrinsic catalytic activity. This was related to the **loop** allowing the pos. charge of **Lys300(c143)** to transiently interact with **Asp355(c194)**, thereby inducing an active conformation of the protease domain. To further test this hypothesis, the charge at position 300(c143) and the **flexibility of the loop** were altered using site-directed mutagenesis designed according to a computer model to affect the interaction between **Lys300(c143)** and **Asp355(c194)**. When the charge at **Lys300(c143)** but not **Lys313(c156)** was reduced, a significant reduction in the intrinsic catalytic activity occurred. Similarly, when the **flexibility (wobbliness)** of the **loop** was enhanced reducing the size of side-chain, the intrinsic catalytic activity was also reduced. By contrast, when the **loop** was made less **flexible**, the intrinsic catalytic activity was increased. These findings were consistent with the hypothesis. The effects of these **mutations** on 2-chain activity were less and often discordant with the intrinsic catalytic activity, indicating that they can be modulated independently. This structure-function disparity can be exploited to create a more zymogenic **prourokinase** (lower intrinsic catalytic activity) with a high catalytic activity, as exemplified by 2 of the **mutants**. The changes in intrinsic catalytic activity and 2-chain activity induced by the **mutations** were due to changes in **kcat** rather than **Km**. Some significant structure-function differences between **prourokinase** and its highly homologous counterpart, tissue plasminogen activator, were also found.

IT 82657-92-9, **Prourokinase**

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study) (identification of a **flexible loop** region (297-313) in human **urokinase-type plasminogen activator** which helps determine its catalytic activity)

RETABLE

Referenced Author (RAU)	Year (R PY)	VOL (R VL)	PG (R PG)	Referenced Work (RWK)	Referenced File
Bugge, T	1996	87	709	Cell	HCAPLUS
Gurewich, V	1989	82	1956	J Clin Invest	
Huber, A	1978	11	114	Acc Chem Res	
Lamba, D	1996	258	117	J Mol Biol	HCAPLUS
Lijnen, H	1990	265	5232	J Biol Chem	HCAPLUS
Liu, J	1992	31	6311	Biochemistry	HCAPLUS
Liu, J	1996	35	14070	Biochemistry	HCAPLUS
Liu, J	1993	81	980	Blood	HCAPLUS
Liu, J	1992	267	15289	J Biol Chem	HCAPLUS
Liu, J	1995	270	8408	J Biol Chem	HCAPLUS
Liu, J	1991	88	2012	J Clin Invest	HCAPLUS
Madison, E	1993	262	419	Science	HCAPLUS
Moscatelli, D	1988	948	67	Biochim Biophys Acta	HCAPLUS
Orini, G	1991	195	691	Eur J Biochem	
Pannell, R	1986	67	1215	Blood	HCAPLUS
Pannell, R	1987	69	22	Blood	HCAPLUS
Pannell, R	1988	81	853	J Clin Invest	HCAPLUS
Petersen, L	1990	29	3451	Biochemistry	HCAPLUS
Petersen, L	1988	263	11189	J Biol Chem	HCAPLUS
Rijken, D	1982	257	2920	J Biol Chem	HCAPLUS
Romer, J	1994	102	519	J Invest Dermatol	MEDLINE
Sappino, A	1989	109	2471	J Cell Biol	HCAPLUS
Spraggan, G	1995	3	681	Structure	HCAPLUS
Strickland, S	1976	9	231	Cell	HCAPLUS
Tachias, K	1996	271	28749	J Biol Chem	HCAPLUS
Tachias, K	1997	272	28	J Biol Chem	HCAPLUS
Tate, K	1986	26	338	Biochemistry	

Valinsky, J	1981	25	471	Cell	HCAPLUS
Voskuilen, M	1987	262	5944	J Biol Chem	HCAPLUS
Winkler, M	1986	25	4041	Biochemistry	HCAPLUS

L98 ANSWER 29 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1997:400620 HCAPLUS
 DN 127:132590
 TI Dissection of intrinsic catalytic activity of pro-urokinase
 AU Wu, H.; Sun, Z. Y.; Liu, B. F.; Ma, Z.; Xue, Y. M.; Tang, W.; Jiang, Y. P.; Gurewich, V.; Zhu, D. X.; Liu, J. N.
 CS Vascular Research Laboratory, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02215, USA
 SO Protein Engineering (1997), 10(Suppl.), 70
 CODEN: PRENE9; ISSN: 0269-2139
 PB Oxford University Press
 DT Journal
 LA English
 AB Site-directed mutagenesis of prourokinase proved Lys300 is a key structural determinant in its activity. The flexible nature of the loop made up of amino acids 297-313 allowed Lys300 to form a salt bridge with Asp335 which generated most of the intrinsic catalytic activity.
 IT 82657-92-9, Prourokinase
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
 (site-directed mutagenesis of prourokinase catalytic activity is generated by Lys300 forming a salt bridge with Asp335)

RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
Liu, J	1996			Biochemistry	
Madioson, E	1993	262	419	Science	

L98 ANSWER 30 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1997:316029 HCAPLUS
 DN 127:46884
 TI Mutation of Arg154 to Gly154 in urokinase augments its fibrin-specificity
 AU Peng, Guihong; Ma, Zhong; Kuai, Letian; Zhu, Dexu
 CS Dep. Biochem. and Natl. Lab. Pharmaceutical Biotechnology, Nanjing Univ., Nanjing, 210093, Peop. Rep. China
 SO Biochemistry and Molecular Biology International (1997), 41(5), 887-894
 CODEN: BMBIES; ISSN: 1039-9712
 PB Academic
 DT Journal
 LA English
 AB Recombinant single-chain urokinase -type plasminogen activator (rscu-PA) and its mutant constructed by in vitro site specific mutagenesis of Arg154 in rscu-PA to Gly154 (mscu-PA) were both expressed in *Escherichia coli*. After in vitro denaturation and renaturation, the rscu-PA and mscu-PA were purified to homogeneity by Zn²⁺ selective precipitation, anti-u-PA IgG-sepharose CL4B affinity chromatog. After activation by plasmin, the kinetic consts. for the resultant mtcu-PA against synthetic substrate S2444 hydrolysis were found to be essentially identical to recombinant 2-chain urokinase-type plasminogen activator (rtcu-PA), suggesting that no impairment had been exerted on the catalytic active site of mtcu-PA. However, both 125I-fibrin plasma-clot lysis and fibrinogenolysis showed that mtcu-PA possessed a higher fibrinolytic

activity but hardly any degradation of fibrinogen in plasma compared to rtcu-PA and rscu-PA. Thus, the substitution of Arg154 by Gly154 in tcu-PA promoted the fibrin-specificity of urokinase.

RETABLE

Referenced (RAU)	Author	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
Bennett, J		1982	257	8049	J Biol Chem	HCAPLUS
Bradford, M		1976	72	248	Anal Biochem	HCAPLUS
Graffney, J		1990	64	398	Health Thromb Haemos	
Gurewich, V		1992	667	224	Annals of the New Yo	MEDLINE
Husain, S		1993	268	8575	J Biol Chem	
John, R		1990	27	6374	Biochemistry	
Laemmli, U		1970	227	680	Nature	HCAPLUS
Lijnen, H		1987		359	Eur J Biochem	HCAPLUS
Lijnen, H		1986	261	1253	J Biol Chem	HCAPLUS
Liu, J		1993	81	980	Blood	HCAPLUS
Liu, J		1991	10	1035	Science in China (se	
Ma, Z		1996	39	523	Science in China(ser	HCAPLUS
Nelles, L		1987	262	5682	J Biol Chem	HCAPLUS
Ploug, T		1957	24	278	Biochem Biophys Acta	
Rampling, M		1976	678	43	Clin chem Acta	
Sambrook, J		1989			Molecular cloning, A	
Song, A		1992	35	966	Science in China Ser	HCAPLUS
Stump, D		1986	261	17120	J Biol Chem	HCAPLUS
Valery, N		1995	270	8680	J Biol Chem	
Verstraete, M		1986	67	1529	Blood	
Wrinkler, M		1986	25	4041	Biochemistry	HCAPLUS

L98 ANSWER 31 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1996:726431 HCAPLUS

DN 126:3632

TI **Urokinase mutant with better fibrin-specificity**

AU Ma, Zhong; Yu, Ruirong; Hua, Zichun; Zhu, Dexu

CS Department Biochemistry, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Science in China, Series C: Life Sciences (1996), 39(5), 523-533

CODEN: SCCLFO; ISSN: 1006-9305

PB Science in China Press

DT Journal

LA English

AB A 150-156 amino acid-deleted **single-chain** urokinase-type plasminogen activator (dscu-PA) and its recombinant wild-type counterpart (rscu-PA) were both expressed in *Escherichia coli*. After denaturation and renaturation in vitro, the expressed products were both purified to a single silver-stained band by means of IgG affinity chromatog. After activation by plasmin, similar enzymic consts. based on the hydrolysis of synthetic substrate S2444 by the two-chain mol. forms of dscu-PA and rscu-PA, or native tcu-PA were observed, suggesting that no impairment had been exerted on the catalytic active site of dtcu-PA by the 150-156 amino acids deletion. In both in vitro fibrin-clot and 125I-fibrin sepharose lysis tests, dtcu-PA showed a significantly higher fibrinolytic activity than rtcu-PA or rscu-PA. Hardly any effect on the concentration of fibrinogen in plasma was found in dtcu-PA. It was concluded that dtcu-PA had a higher fibrin specificity and that tcu-PA could be provided with better fibrin specificity by means of mutation.

IT 82657-92-9, **Single-chain urokinase**
-type plasminogen activator

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(development of urokinase-type plasminogen activator
mutant with better fibrin-specificity)

L98 ANSWER 32 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1996:632233 HCAPLUS
 DN 125:295880
 TI A site-directed mutagenesis of pro-urokinase
 which substantially reduces its intrinsic activity
 AU Liu, Jian-Ning; Tang, Wei; Sun, Zi-Yong; Kung, Wendy; Pannell, Ralph;
 Sarmientos, Paolo; Gurewich, Victor
 CS Institute for the Prevention of Cardiovascular Disease, Harvard Medical
 School, Boston, MA, 02215, USA
 SO Biochemistry (1996), 35(45), 14070-14076
 CODEN: BICHAW; ISSN: 0006-2960
 PB American Chemical Society
 DT Journal
 LA English
 AB Single-chain urokinase-type plasminogen activator or pro-urokinase is a zymogen with an intrinsic catalytic activity which is greater than that of most other zymogens. To study the structural basis for this activity, a three-dimensional homol. model was calculated using the crystallog. structure of chymotrypsinogen, and the structure-function relationship was studied using site-directed mutagenesis and kinetic anal. This model revealed a unique Lys300 in pro-urokinase which could form a weak interaction with Asp355, adjacent to the active site Ser356. It was postulated that this lysine, by its ε-amino group, may serve to pull Ser356 close to the active position, thereby inducing the higher intrinsic activity of pro-urokinase. This was consistent with the published finding that a homologous lysine (Lys416) in single chain tissue plasminogen activator when mutated to serine induced some reduction in activity. To test this hypothesis, a site-directed mutant with a neutral residue (Lys300 → Ala) was produced and characterized. The Ala300-pro-urokinase had a 40-fold lower amidolytic activity than that of pro-urokinase. It was also stable in plasma at much higher concns. than pro-urokinase, reflecting much attenuated plasminogen activation. Plasmin activatability was comparable to that of pro-urokinase, but the resultant two-chain derivative (Ala300-urokinase) had a lower enzymic activity (~33% that of urokinase) due to a reduction of kcat. Interestingly, the KM of two-chain Ala300-urokinase against plasminogen was 5.8-fold lower than that of urokinase, being similar to that of pro-urokinase which has a KM about 5-fold lower than urokinase. In conclusion, the hypothesis that Lys300 is a key structural determinant of the high intrinsic activity of pro-urokinase was confirmed by these studies. This residue also appears to be important for the full expression of the enzymic activity of urokinase.
 IT 56-87-1, Lysine, biological studies
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
 (position 300; site-Directed Mutagenesis of Pro-urokinase which Substantially Reduces Its Intrinsic Activity)
 IT 82657-92-9, Single-chain urokinase
 -type plasminogen activator
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
 (site-Directed Mutagenesis of Pro-urokinase
 which Substantially Reduces Its Intrinsic Activity)

L98 ANSWER 33 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1996:392791 HCAPLUS
 DN 125:106288

TI High expression of human **pro-urokinase** promoted by
 T7 promoter in **Escherichia coli**
 AU Dong, Chen; Chen, Xuaochun; Chen, Yuhong; Ma, Zhong; Xia, Yan; Hua, Zichun
 CS Department of Biochemistry, Nanjing University, Nanjing, 210093, Peop.
 Rep. China
 SO Nanjing Daxue Xuebao, Ziran Kexue (1995), 31(4), 606-610
 CODEN: NCHPAZ; ISSN: 0469-5097
 PB Nanjing Daxue
 DT Journal
 LA Chinese
 AB **Pro-urokinase (Pro-UK)**, the second generation of thrombolytic agent, was superior to **urokinase** by its more specific affinity to fibrin and mild enzymic activity, which meant that it had less tendency to cause hemorrhage when injected in a large dose. But because of its rare natural sources, insufficient **Pro-UK** would be available for clin. therapy unless it was produced by genetic engineering methods. Up to now, the expression level of **Pro-UK** cDNA in **E. coli** has hardly been more than 2% of total bacterial proteins. In this article, a human **Pro-UK** cDNA controlled by **T7** promoter was reported to be successfully expressed in **E. coli** with 18% expression level. To create **pET3d/Pro-UK** cDNA, a fragment of **Pro-UK** gene cleaved from **pUC9/Pro-UK** plasmid by **HindIII** (Klenow blunted) and **BspHI**, was inserted into the **pET3d** expression vector, which was cut with **BamHI** (Klenow blunted) and **NcoI**. The pos. **recombined** plasmid was confirmed by cutting with several restriction enzymes, and was transformed into **E. coli BL21(DE3)**. Following induction by 1 mmol/L IPTG, **Pro-UK** cDNA was expressed with an extra band of 43 kDa on SDS-PAGE, and the band was proved to be human **Pro-UK** by western blotting anal. Densitometric scanning revealed that the expression level of **Pro-UK** was 18% of total cellular protein. The expression bacteria were sonicated and the resultant lysate and sediment were collected sep. After denaturation and renaturation in vitro, the sediment part displayed fibrinolytic activity of about 300,000 IU/L culture medium, whereas the supernatant rarely had the same activity. This suggests that the expression product of **pET3d/Pro-UK** was mainly in the form of inclusion bodies.
 IT 82657-92-9P, **Prourokinase**
 RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
 (high expression of recombinant human prourokinase promoted by T7 promoter in **Escherichia coli**)

L98 ANSWER 34 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1996:176193 HCPLUS
 DN 124:230074
 TI Renaturation of recombinant human **pro-urokinase** expressed in **Escherichia coli**
 AU Hua, Zi-Chun; Dong, Chen; Zhu, De-Xu
 CS Pharmaceutic Biotechnology Key Lab., Nanjing Univ., Nanjing, 210093, Peop.
 Rep. China
 SO Biochemical and Biophysical Research Communications (1996), 220(1), 131-6
 CODEN: BBRCA9; ISSN: 0006-291X
 PB Academic
 DT Journal
 LA English
 AB A synthetic gene encoding human **pro-urokinase** (**pro-UK**) with **E. coli**-favored codon usage was cloned into plasmid **pET-3d** and expressed in **E.**

coli BL21(DE3) LysS strain. The expressed products, which accumulated as inactive inclusion bodies, were denatured and renatured in vitro. A broad range of parameters such as pH, protein concentration, denaturant concentration, the use of cosolvent polyethylene glycol and presence of basic or acidic amino acid was examined. At optimal renaturation condition, pro-**u-UK** activity of more than 1000 I.U. was obtained from 1 mL cell culture.

IT 82657-92-9P, Pro-urokinase
 RL: BMF (Bioindustrial manufacture); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)
 (renaturation of recombinant human pro-urokinase expressed in **Escherichia coli**)

L98 ANSWER 35 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1995:796989 HCPLUS
 DN 123:224082
 TI Native and non-glycosylated recombinant single-chain urokinase-type plasminogen activator are recognized by different receptor systems on rat parenchymal liver cells
 AU van der Kaaden, Marieke E.; Rijken, Dingeman C.; Groeneveld, Eleonore; van Berkel, Theo J. C.; Kuiper, Johan
 CS Sylvius Lab., Univ. Leiden, Leiden, Neth.
 SO Thrombosis and Haemostasis (1995), 74(2), 722-9
 CODEN: THHADQ; ISSN: 0340-6245
 PB Schattauer
 DT Journal
 LA English
 AB The recognition systems mediated the clearance of glycosylated high-mol.-weight single-chain urokinase-type plasminogen activator (HMW-scu-PA, produced in human embryonic kidney cells) and recombinant non-glycosylated scu-PA (rscu-PA, produced in **E. coli**) were analyzed by studying their binding characteristics to freshly isolated rat parenchymal liver cells. The binding of ^{125}I -HMW-scu-PA at 4° was calcium-dependent and of high affinity (K_d = 37.6 nM) and could be inhibited by low mol. weight two-chain u-PA (LMW-tcu-PA) and lactose, but not by the low d. lipoprotein receptor-related protein (LRP)-associated 39-kDa protein (RAP), rscu-PA or a mutant form lacking amino acids 11-135 (Delta 125-rscu-PA). Removal of the carbohydrate side chain of HMW-scu-PA by treatment with N-glycosidase F, completely reduced the specific binding to the parenchymal cells and strongly reduced its competition with ^{125}I -HMW-scu-PA in cell binding. Recombinant scu-PA also bound with high affinity (K_d = 38.7 nM) to the parenchymal liver cells. The binding of ^{125}I -rscu-PA could be competed for by unlabeled rscu-PA while Delta 125 125-rscu-PA, LMW-tcu-PA or lactose were ineffective. In contrast to HMW-scu-PA, binding of ^{125}I -rscu-PA could be effectively inhibited by RAP (K_i = 1.1 nM), while also its association and degradation, as determined at 37°, were inhibited by RAP. Pretreatment of the parenchymal cells with proteinase K supplied further evidence for the involvement of two different receptor systems. The binding of rscu-PA was decreased for 91%, while that of HMW-scu-PA showed a decrease of 51%. It is suggested that native HMW-scu-PA is bound and degraded by the rat parenchymal liver cells via a lectin-like recognition site, while non-glycosylated recombinant scu-PA is bound and degraded by rat parenchymal liver cells via the low d. lipoprotein receptor-related protein (LRP). The differences in recognition system for native and recombinant proteins by liver cells suggest that the glycosylation of recombinant proteins, as obtained in mammalian expression systems, can be important for their physiol. fate and their

IT pharmacol. application.

IT 82657-92-9, Single-chain urokinase
 -type plasminogen activator 82657-92-9D, Single-chain urokinase-type plasminogen activator, nonglycosylated

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
 (native and nonglycosylated recombinant single-chain urokinase-type plasminogen activator are recognized by different receptor systems on rat parenchymal liver cells)

L98 ANSWER 36 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1995:419764 HCPLUS
 DN 123:2186

TI Use of bacteriophage T7 RNA polymerase to direct expression of cloned pro-urokinase gene in *Escherichia coli*

AU Sui, Guangchao; Liu, Fang; Hu, Meihao
 CS College Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China
 SO Beijing Daxue Xuebao, Ziran Kexueban (1994), 30(6), 728-33
 CODEN: PCTHAP; ISSN: 0479-8023
 PB Beijing Daxue Chubanshe
 DT Journal
 LA Chinese
 AB Human pro-urokinase (pro-UK) was produced in *Escherichia coli*. This was done using the T7 gene .vphi.10 promoter on the vector pET3c. After induction with IPTG, the pro-UK was expressed with an expression level of about 1600 IU/L of cell culture assayed on a fibrin plate after a denaturation-refolding procedure. The product was characterized by Western blot anal. The blot displayed a major band at 43 kDa slightly lower than natural 54 kDa pro-UK, probably due to the lack of glycosylation of pro-UK in *E. coli*.

IT 82657-92-9P, Prourokinase
 RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
 (use of bacteriophage T7 RNA polymerase to direct expression of cloned pro-urokinase gene in *Escherichia coli*)

L98 ANSWER 37 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1994:571656 HCPLUS
 DN 121:171656

TI Expression of a fibrinolytically active human pro-urokinase fusion protein in *Escherichia coli*

AU Hua, Zichun; Jie, Li; Zhu, Dexu
 CS Department Biochemistry, Nanjing University, Nanjing, 210008, Peop. Rep. China
 SO Biochemistry and Molecular Biology International (1994), 33(6), 1215-20
 CODEN: BMBIES; ISSN: 1039-9712
 DT Journal
 LA English
 AB The gene encoding human pro-urokinase (pro-UK) was cloned into plasmid pEZ318 and fused to the gene coding for the signal peptide of staphylococcal protein A and IgG binding domain. The fusion protein which was synthesized under the control of the T7 promoter in *Escherichia coli* and secreted into the growth medium was found to be fibrinolytically active. Approx. 60% of the total activity was secreted into the culture medium, where

levels of activity approached 150,000 I.U./L and about 40% of the total activity remained in the cell lysate with levels of activity around 100,000 I.U./L. The fusion protein was purified in a single step by IgG affinity chromatog. These results demonstrate that human pro-UK can be synthesized and secreted by *E. coli* as a fibrinolytically active fusion protein.

IT 82657-92-9, Pro-urokinase

RL: BIOL (Biological study)
(gene for, of human, cloning of, in *Escherichia coli*)

L98 ANSWER 38 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1994:553198 HCPLUS

DN 121:153198

TI A methylotrophic and glucotrophic **mutant** strain for producing a heterologous protein

IN Okabayashi, Ken; Ohmura, Takao; Yokoyama, Kazumasa; Kawabe, Haruhide

PA Green Cross Corp., Japan

SO Eur. Pat. Appl., 13 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 606917	A2	19940720	EP 1994-100460	19940113 <--
	EP 606917	A3	19950719		
	R: BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
	JP 06209763	A2	19940802	JP 1993-4289	19930113 <--
	US 5643792	A	19970701	US 1994-181242	19940113 <--
PRAI	JP 1993-4289	A	19930113	<--	

AB A methylotrophic and glucotrophic **mutant** strain and its use for producing a heterologous protein are disclosed. The **mutant** strain can be grown in a medium containing both methanol and glucose, with the effect that the growth of the strain and production of a heterologous protein proceed at the same time. Accordingly, a heterologous protein can be produced in a large amount in a short time. *Pichia pastoris* GCP104 (containing human serum albumin expression cassette under the control of AOX1 promoter) having AOX1 gene deleted was used to select strain GCP101 that exhibits **mutation** on the AOX2 promoter and, as a result, efficient utilization of MeOH. Strain GCP101 was then EMS-**mutated** to select **mutant** ECCR72, that was capable of utilizing both glucose and MeOH and produce an increased amount of HSA.

IT 82657-92-9P, Prourokinase

RL: PREP (Preparation)

(manufacture of, with methylotrophic and glucotrophic **mutant**)

L98 ANSWER 39 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1994:100542 HCPLUS

DN 120:100542

TI Protease-resistant **urokinases**, their preparation with recombinant cells, and their use in pharmaceuticals

IN Blaber, Michael; Heyneker, Herbert L.; Vehar, Gordon A.

PA Genentech, Inc., USA

SO U.S., 12 pp. Cont.-in-part of U.S. Ser. No. 725,468, abandoned.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 5

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5219569	A	19930615	US 1985-766858	19850816 <--
	DK 8601813	A	19861023	DK 1986-1813	19860421 <--

DK 175304	B1	20040816		
EP 200451	A1	19861105	EP 1986-302981	19860421 <--
EP 200451	B1	19931215		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL				
HU 40696	A2	19870128	HU 1986-1656	19860421 <--
HU 202586	B	19910328		
DD 251791	A5	19871125	DD 1986-289442	19860421 <--
ZA 8602976	A	19871230	ZA 1986-2976	19860421 <--
DD 258026	A5	19880706	DD 1986-289443	19860421 <--
DD 264939	A5	19890215	DD 1986-310554	19860421 <--
AT 68825	E	19911115	AT 1986-302980	19860421 <--
AT 98686	E	19940115	AT 1986-302981	19860421 <--
ES 554249	A1	19871116	ES 1986-554249	19860422 <--
US 5073494	A	19911217	US 1990-522480	19900511 <--
US 5147643	A	19920915	US 1991-741120	19910805 <--
US 5756093	A	19980526	US 1994-275335	19940714 <--
US 5714372	A	19980203	US 1994-306928	19940915 <--
PRAI	US 1985-725468	B2	19850422	<--
	US 1985-766858	A	19850816	<--
	US 1986-846697	A	19860401	<--
	EP 1986-302980	A	19860421	<--
	EP 1986-302981	A	19860421	<--
	US 1987-71506	B1	19870709	<--
	US 1988-186494	B1	19880426	<--
	US 1990-522480	A3	19900511	<--
	US 1991-741120	A2	19910805	<--
	US 1991-808366	B1	19911216	<--
	US 1991-808537	B1	19911216	<--
	US 1993-101276	B1	19930802	<--
	US 1993-126114	B1	19930923	<--

AB Single-chain human **urokinase** analogs modified at residues **Lys**-135 or -136 and/or at Arg-156 to **Lys**-158 are resistant to proteolytic cleavage. [.DELTA.**Lys**-136,Phe-157, **Lys**-158]human **urokinase** was produced with recombinant **E. coli**, purified, and tested for plasmin resistance. The analog retained most of the plasminogen activating capacity and was far less susceptible to plasmin activation than was the recombinant native single-chain human **urokinase**.

L98 ANSWER 40 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1993:554583 HCPLUS

DN 119:154583

TI Implication of cysteine residues in the activity of **single-chain urokinase-plasminogen activator**

AU Hamelin, Jocelyne; Sarmientos, Paolo; Orsini, Gaetano; Galibert, Francis

CS Cent. Hayem, Hop. Saint-Louis, Paris, 75475, Fr.

SO Biochemical and Biophysical Research Communications (1993), 194(2), 978-85

CODEN: BBRCA9; ISSN: 0006-291X

DT Journal

LA English

AB Single-chain **urokinase**-type plasminogen

activator (I) contains 24 cysteine residues involved in 12 disulfide bonds and distributed all along the 3 domains of the protein. In order to investigate the role of these disulfide bridges in the catalytic activities of I, site-specific **mutagenesis** was used to construct 10 mutants in which some cysteine residues were changed to serine residues. Each **mutated** DNA fragment was cloned into a prokaryotic expression vector and the protein expressed in **E. coli**. **Mutant** proteins of the expected size were produced and analyzed for amidolytic and fibrinolytic activities. From

*No TX done +
before +
start*

this, it was shown that: (1) the disulfide bonds in the epidermal growth factor (EGF)-like and in the kringle domains are not necessary. Moreover, disulfide bond deletion in the kringle domain improved those catalytic activities; (2) on the contrary, the disulfide bridges in the catalytic domain are essential for maintaining both activities.

L98 ANSWER 41 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1993:447380 HCAPLUS
 DN 119:47380
 TI The pro-urokinase molecule: Structural and biotechnological aspects
 AU Sarmientos, Paolo; Lansen, Jacqueline; Mazue, Guy; Carminati, Paolo; Roncucci, Romeo
 CS Farmitalia Carlo Erba, Milan, Italy
 SO Biotec (Brescia) (1990), 5(2), 61-3, 65-7
 CODEN: BBRCED; ISSN: 0393-9146
 DT Journal; General Review
 LA English/Italian
 AB A review with no refs. of the authors' work on the structure, production, and potential as a thrombolytic agent of human **single-chain** urokinase-type plasminogen activator and a low-mol.-weight derivative obtained by site-directed mutagenesis.
 IT 82657-92-9
 RL: BIOL (Biological study)
 (structural and biotechnol. aspects of)

L98 ANSWER 42 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1993:421552 HCAPLUS
 DN 119:21552
 TI High expression vectors for the production of recombinant single-chain urinary plasminogen activator from **Escherichia coli**
 AU Brigelius-Flohe, Regina; Steffens, Gerd; Strassburger, Wolfgang; Flohe, Leopold
 CS Cent. Res., Gruenenthal G.m.b.H., Aachen, W-5100, Germany
 SO Applied Microbiology and Biotechnology (1992), 36(5), 640-9
 CODEN: AMBIDG; ISSN: 0175-7598
 DT Journal
 LA English
 AB An expression cassette containing a synonymous gene for human **single** -chain urokinase-type plasminogen activator (Rscu-PA) 5'-flanked by a trp promoter and the **Shine-Dalgarno** sequence of the xyl A operon of Bacillus subtilis and terminated by the terminators trp A and Tn10 was constructed and inserted into a pBR322 derivative to yield pBF160. When compared to pUK54 trp 207-1 containing the natural scuPA gene without the **Shine-Dalgarno** sequence and terminator, the expression efficiency of pBF160 in **Escherichia coli** strains was improved by one order of magnitude. Replacement of the trp by the tac promoter (pBF171) did not affect expression. Inserting the **Shine-Dalgarno** sequence and Tn10 terminator into pUK54 trp 207-1 (pWH1320) slightly increased the expression level, whereas elimination of the **Shine-Dalgarno** sequence and the terminators from pBF160 with almost complete conservation of the synonymous structural gene (pBF191) significantly reduced the expression. Variation of the distance between the **Shine-Dalgarno** sequence and the start codon between 8 and 10 bp (pBF163) proved irrelevant. In conclusion, poor expression of mammalian genes in **E. coli** may result from both improperly designed regulatory elements and structural features of the coding region and therefore de-novo synthesis of the gene may be required to obtain satisfactory expression.
 IT 146481-75-6 146481-76-7
 RL: PRP (Properties); BIOL (Biological study)

(nucleotide sequence of)

IT 146481-77-8

RL: PRP (Properties); BIOL (Biological study)
(nucleotide sequence of, complete)

L98 ANSWER 43 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1993:119887 HCAPLUS

DN 118:119887

TI Plasminogen activator analogs and their preparation using microorganisms
IN Steffens, Gerd J.; Guenzler, Wolfgang A.; Flohe, Leopold; Brigelius-Flohe,
Regina E.; Wolf, Bernard

PA Gruenenthal GmbH, Germany

SO Ger. Offen., 33 pp.

CODEN: GWXXBX

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 4101736	A1	19920723	DE 1991-4101736	19910122 <--
	RU 2108387	C1	19980410	RU 1992-5010518	19920109 <--
	HU 63877	A2	19931028	HU 1992-127	19920115 <--
	HU 212510	B	19960729		
	EP 496327	A1	19920729	EP 1992-100864	19920120 <--
	EP 496327	B1	20010816		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, PT, SE				
	AT 204326	E	20010915	AT 1992-100864	19920120 <--
	ES 2164048	T3	20020216	ES 1992-100864	19920120 <--
	PT 496327	T	20020228	PT 1992-100864	19920120 <--
	FI 9200263	A	19920723	FI 1992-263	19920121 <--
	JP 06169770	A2	19940621	JP 1992-8765	19920121 <--
	LT 3948	B	19960527	LT 1993-1532	19931206 <--
	HK 1010108	A1	20020404	HK 1998-110955	19980925 <--

PRAI DE 1991-4101736 A 19910122 <--

OS MARPAT 118:119887

AB Analogs of single-chain urinary plasminogen activator (scu-PA) with modified terminal regions (Ser-X1-X2-scu-PA143-403-Z-Leu-Ala-LeuOH (I) (X1 = Asn, Pro, Ser; X2 = bond, Glu, Pro-Glu, Pro-Pro-Glu, Glu-Leu-His-Leu-Leu-Gln-Val-Pro-Ser-Asn; Z = Lys -Glu-Glu-Asn-Gly, Arg-Gly-Asp-Ser-Pro; scu-PA143-403 = unglycosylated amino acid sequence from Glu143 to Thr403 of single-chain 54,000-Da urokinase-type PA) are manufactured for use as thrombolytics by expression of the gene in a microbial host. I are not bound and inactivated by specific cellular urokinase receptors. I (Z = Arg-Gly-Asp-Ser-Pro) show a marked affinity for blood platelets; this provides for concentration of I at its site of action. Scu-PA gene fragments were incorporated into a pBR322 derivative along with a synthetic multicloning site, a transcription terminator, and a tac promoter to provide plasmid pGRTac06 for expression in *Escherichia coli*. The protein product was refolded and cleaved to form active 2-chain PA.

IT 146411-89-4

RL: BIOL (Biological study)
(nucleotide sequence and expression in *Escherichia coli* of)

IT 146411-73-6 146411-74-7 146411-75-8

146411-76-9 146411-77-0

RL: PRP (Properties); BIOL (Biological study)
(nucleotide sequence of, urokinase analog genes containing)

L98 ANSWER 44 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1993:36558 HCAPLUS

DN 118:36558

TI The influence of glycosylation on the catalytic and fibrinolytic properties of **pro-urokinase**
 AU Lenich, Catherine; Pannell, Ralph; Henkin, Jack; Gurewich, Victor
 CS Inst. Prevent. Cardiovasc. Dis., New England Deaconess Hosp., Boston, MA,
 USA
 SO Thrombosis and Haemostasis (1992), 68(5), 539-44
 CODEN: THHADQ; ISSN: 0340-6245
 DT Journal
 LA English
 AB The authors previously found that human **pro-urokinase** (**pro-UK**) expressed in **Escherichia coli** is more active in fibrinolysis than **recombinant** human **pro-UK** obtained from mammalian cell culture media. To determine whether this difference is related to the lack of glycosylation of the **E. coli** product, the authors compared the activity of **E. coli**-derived **pro-UK** [(-)**pro-UK**] with that of a glycosylated **pro-UK** [(+)**pro-UK**] and of a mutant of **pro-UK** missing the glycosylation site at Asn-302 [(-)(302)**pro-UK**]. The latter two **pro-UKs** were obtained by expression of the human gene in a mammalian cell. The nonglycosylated **pro-UKs** were activated by plasmin more efficiently (~2-fold) and were more active in clot lysis (1.5-fold) than the (+)**pro-UK**. Similarly, the nonglycosylated two-chain derivs. (**UKs**) were more active against plasminogen and were more rapidly inactivated by plasma inhibitors than the (+)**UK**. These findings indicate that glycosylation at Asn-302 influences the activity of **pro-UK/UK** and could be the major factor responsible for the enhanced activity of **E. coli**-derived **pro-UK**.
 IT 82657-92-9, **Pro-urokinase**
 RL: BIOL (Biological study)
 (catalytic and fibrinolytic activity of, of human, glycosylation influence on)

L98 ANSWER 45 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1993:17442 HCAPLUS
 DN 118:17442
 TI Palindromic DNA sequence for enhanced gene expression in **Escherichia coli**
 IN Miyake, Toshio; Yamada, Masayuki; Sakaguchi, Reiko; Kubo, Miki
 PA Tosoh Corp., Japan
 SO Jpn. Kokai Tokkyo Koho, 8 pp.
 CODEN: JKXXAF

DT Patent
 LA Japanese
 FAN.CNT 1
 PATENT NO. KIND DATE APPLICATION NO. DATE
 ----- ----- ----- -----
 PI JP 04004884 A2 19920109 JP 1990-102848 19900420 <--
 PRAI JP 1990-102848 19900420 <--
 AB A palindromic DNA sequence useful in stabilizing mRNA transcribed in **E. coli** is provided to increase the yield of protein products. Production of human **prourokinase** derivative by **E. coli** was greatly enhanced by inserting a synthetic palindromic DNA sequence between the tac promoter and the **Shine-Dalgarno** sequence.

L98 ANSWER 46 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1992:483570 HCAPLUS
 DN 117:83570
 TI Structure-function relationship of basic fibroblast growth factor: site-directed **mutagenesis** of a putative heparin-binding and

receptor-binding region
 AU Presta, M.; Statuto, M.; Isacchi, A.; Caccia, P.; Pozzi, A.; Gualandris, A.; Rusnati, M.; Bergonzoni, L.; **Sarmientos, P.**
 CS Sch. Med., Univ. Brescia, Brescia, 25123, Italy
 SO Biochemical and Biophysical Research Communications (1992), 185(3), 1098-107
 CODEN: BBRCA9; ISSN: 0006-291X
 DT Journal
 LA English
 AB Basic residues Arg-118, Lys-119, Lys-128, and Arg-129 within a putative heparin-binding and receptor-binding region of the 155-amino acid form of basic fibroblast growth factor (bFGF) have been changed to neutral glutamine residues by site-directed mutagenesis of the human bFGF cDNA. The bFHF mutant (M6B-bFGF) was expressed in *E. coli* and purified to homogeneity. When compared to wild type bFGF, M6B-bFGF showed in cultured endothelial cells a similar receptor-binding capacity and mitogenic activity, but a reduced affinity for heparin-like low affinity binding sites, a reduced chemotactic activity, and a reduced capacity to induce the production of urokinase -type plasminogen activator. In vivo, M6B-bFGF lacked a significant angiogenic activity. Modifications of both the primary and the tertiary structure of bFGF appear to be responsible for the modified biol. properties of M6B-bFGF, thus confirming the possibility to dissociate at the structural level some of the biol. activities exerted by bFGF on endothelial cells.

L98 ANSWER 47 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1992:446699 HCAPLUS
 DN 117:46699
 TI Amidated human prourokinase and its manufacture by enzymic modification of the protein
 IN Gozzini, Luigia; Visco, Carlo; Perego, Rita; Roncucci, Romeo; **Sarmientos, Paolo**
 PA Farmitalia Carlo Erba S.r.l., Italy
 SO Ger. Offen., 17 pp.
 CODEN: GWXXBX
 DT Patent
 LA German
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 4122688	A1	19920116	DE 1991-4122688	19910709 <--
	GB 2246133	A1	19920122	GB 1991-14846	19910710 <--
	JP 04252185	A2	19920908	JP 1991-196960	19910711 <--
PRAI	GB 1990-15369	A	19900712 <--		
AB	C-terminal amidated human prourokinase (hPUNKH2) is prepared by expressing a gene for hPUK-Gly-Yn (n=1-4; Y=basic amino acid) in an appropriate host cell; isolating the recombinant protein; and treating it with carboxypeptidase B and/or an amidating enzyme such as peptidylglycine α -amidating monooxygenase.				
IT	82657-92-9DP, Prourokinase, carboxy terminal-modified RL: PREP (Preparation) (manufacture of human, with transgenic cells, carboxy terminal-amidated prourokinase enzymic manufacture from)				

L98 ANSWER 48 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1992:230758 HCAPLUS
 DN 116:230758
 TI Inhibitor-resistant analogs of prourokinase
 IN Brandazza, Anna; Lansen, Jaqueline; Orsini, Gaetano; **Sarmientos, Paolo**
 PA Farmitalia Carlo Erba S.r.l., Italy
 SO Ger. Offen., 9 pp.

CODEN: GWXXBX

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 4125193	A1	19920206	DE 1991-4125193	19910730 <--
	GB 2247022	A1	19920219	GB 1991-16457	19910730 <--
	JP 04252184	A2	19920908	JP 1991-190333	19910730 <--
PRAI	IT 1990-21178	A	19900802	<--	

AB **Prourokinase** analogs that are resistant to inhibition by plasminogen activator inhibitor 1 (PAI-1) are obtained by substitution of serine residues with acidic ones. Serines at positions 138, 139, or 303 are substituted with Glu or Asp. One such analog (Glu-303 **prourokinase**) was prepared by site-directed mutagenesis of the gene and expression of the gene in *Escherichia coli*. The analog was as active against the test substrate S2390 as the wild-type enzyme. Under conditions where the wild-type enzyme was inhibited 50% by PAI-1 this analog retained 80% of its activity.

IT 82657-92-9D, **Prourokinase**, amino acid substituted analogs

RL: BIOL (Biological study)

(plasminogen activator inhibitor-resistant, manufacture in *Escherichia coli* of)

L98 ANSWER 49 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1992:230644 HCPLUS

DN 116:230644

TI Biochemical properties of recombinant single-chain urokinase-type plasminogen activator mutants with deletion of Asn2 through Phe157 and/or substitution of Cys279 with Ala

AU Lijnen, Henri R.; Li, Xian Kui; Nelles, Luc; Hu, Mei Hao; Collen, Desire
CS Cent. Thromb. Vasc. Res., Univ. Leuven, Louvain, B-3000, Belg.

SO European Journal of Biochemistry (1992), 205(2), 701-9
CODEN: EJBCAI; ISSN: 0014-2956

DT Journal

LA English

AB The contribution of the NH₂-terminal polypeptide chain and of the Cys148-Cys279 interchain SS bond to the enzyme activity of urokinase-type plasminogen activator (u-PA) was studied using site-specific mutagenesis. Recombinant single-chain u-PA (rscu-PA) variants were produced by transfecting CHO cells with cDNA encoding des(Asn2-Phe157)rscu-PA (rscu-PA with deletion of Asn2-Phe157), [Ala279]rscu-PA (rscu-PA with Cys279-Ala mutation) or des(Asn2-Phe157) [Ala279]rscu-PA [des(Asn2-Phe157)rscu-PA with Cys279-Ala mutation]. Des(Asn2-Phe157)rscu-PA, [Ala279]rscu-PA and des(Asn2-Phe157) [Ala279]rscu-PA, purified from conditioned cell culture medium, were obtained as nearly homogeneous single-chain mols. with Mr approx. 30,000, 54,000, and 30,000, and specific fibrinolytic activities on fibrin plates of 860 IU/mg, 43.0 IU/ μ g and 240 IU/mg, resp., compared to 69.0 IU/ μ g for wild-type rscu-PA obtained in the same expression system. The plasminogen-activating potential in a buffer milieu of [Ala279]rscu-PA was somewhat lower than that of rscu-PA, but that of both deletion mutants was virtually abolished. In a human plasma milieu in vitro, consisting of a radiolabeled human plasma clot submerged in plasma, 50% clot lysis in 2 h required 6.5 μ g/mL [Ala279]rscu-PA or 3.4 μ g/mL rscu-PA, whereas with both deletion mutants no significant clot lysis was observed with up to 16 μ g/mL. Treatment of [Ala279]rscu-PA or rscu-PA with plasmin resulted in quant. conversion to two-chain mols. and was associated with an increase in specific amidolytic activity from about 600 IU/mg to 62.5 IU/ μ g for [Ala279]rscu-PA as compared to an increase from about

0.3 IU/ μ g to 75.0 IU/ μ g for rscu-PA. In contrast, no significant amidolytic activity could be generated by treatment of des(Asn2-Phe157)rscu-PA or des(Asn2-Phe157)[Ala279]rscu-PA with plasmin. The u-PA B-chain, isolated from plasmin-treated [Ala279]rscu-PA, had enzymic properties which were comparable to those of rtcu-PA, with respect to specific fibrinolytic activity, amidolytic activity, kinetics of plasminogen activation and clot-lysis activity in a human plasma milieu in vitro. Following bolus injection into hamsters, the plasma clearances were comparable (0.7-1.1 mL/min) for wild-type rscu-PA and for the three truncated rscu-PA mutants. These results indicate that (a) deletion of residues Asn2-Phe157 results in abolition of the enzyme activity of rscu-PA, (b) the interchain SS bond in u-PA is not required for the enzymic activity of scu-PA, (c) all the determinants required for the enzymic activity of two-chain u-PA are contained within the B-chain, and (d) the region comprising residues Asn2-Phe157 of u-PA is not required for the rapid in vivo clearance.

L98 ANSWER 50 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1992:230500 HCPLUS
 DN 116:230500
 TI An enzyme-linked immunosorbent assay for urokinase-type plasminogen activator (u-PA) and mutants and chimeras containing the serine protease domain of u-PA
 AU Declerck, Paul J.; Van Keer, Leen; Verstreken, Maria; Collen, Desire
 CS Cent. Thrombosis Vasc. Res., Univ. Leuven, Louvain, B-3000, Belg.
 SO Thrombosis and Haemostasis (1992), 67(1), 95-100
 CODEN: THHADQ; ISSN: 0340-6245
 DT Journal
 LA English
 AB An ELISA for quantitation of natural and recombinant plasminogen activators containing the serine protease domain (B-chain) of urokinase-type plasminogen activator (u-PA) was developed, based on two murine monoclonal antibodies, MA-4D1E8 and MA-1L3, raised against u-PA and reacting with nonoverlapping epitopes in the B-chain. MA-4D1E8 was coated on microtiter plates and bound antigen was quantitated with MA-2L3 conjugated with horseradish peroxidase. The intra-assay, inter-assay and inter-dilution coeffs. of variation of the assay were 6%, 15% and 9%, resp. Using recombinant single-chain u-PA (rscu-PA) as a standard, the u-PA-related antigen level in normal human plasma was 1.4 ng/mL. The ELISA recognized the following compds. with comparable sensitivity: intact scu-PA [amino acids (AA) 1 to 411], scu-PA-32k (AA 144 to 411), a truncated (thrombin-derived) scu-PA comprising AA 157 to 411, and chimeric t-PA/u-PA mols. including t-PA(AA1-263)/scu-PA(AA144-411), t-PA(AA1-274)/scu-PA(AA138-411) and t-PA(AA87-274)/scu-PA(AA138-411). Conversion of single-chain to two-chain forms of u-PA or inhibition of active two-chain forms with plasminogen activator inhibitor-1 or with the active site serine inhibitor phenyl-methyl-sulfonyl fluoride, did not alter the reactivity in the assay. In contrast, inactivation with α 2-antiplasmin or with the active site histidine inhibitor Glu-Gly-Arg-CH2Cl resulted in a 3- to 5-fold reduction of the reactivity. When purified scu-PA-32k was added to pooled normal human plasma at final concns. ranging from 20 to 1,000 ng/mL, recoveries in the ELISA were between 84 and 110%. The assay was successfully applied for the quantitation of pharmacol. levels of scu-PA and t-PA(AA87-274)/scu-PA(AA138-411) in plasma during exptl. thrombolysis in baboons. Thus the present ELISA, which is specifically dependent on the presence of the serine protease part of u-PA, is useful for measurement of a wide variety of variants and chimeras of u-PA which are presently being developed for improved thrombolytic therapy.

L98 ANSWER 51 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
AN 1992:146703 HCPLUS
DN 116:146703
TI Mutations affecting the activity of urokinase-type
plasminogen activator
AU Davidow, Lance S.; Dumais, Dennis R.; Smyth, Adrienne P.; Greer, Jonathan;
Moir, Donald T.
CS Collab. Res. Inc., Waltham, MA, 02154, USA
SO Protein Engineering (1991), 4(8), 923-8
CODEN: PRENE9; ISSN: 0269-2139
DT Journal
LA English
AB Mutagenesis throughout the single-chain
urokinase-type plasminogen activator (scu-PA)
cDNA mol., followed by expression of the mutant genes and
secretion of the resulting mutant proteins from yeast, has been
used to determine the amino acid residues important for activity of scu
-PA mols. Twelve out of 13 colonies secreting variant
scu-PA mols. with decreased ability to form a zone of
fibrinolysis had mutant genes with a single codon alteration in
the serine protease encoding domain (B-chain). Many of these
changes are of highly conserved residues in the serine proteases and are
consequently of considerable interest. A model three-dimensional
structure of the protease domain of urokinase was used to
explain the basis for the effects of these down mutations. The
model showed that the strongest down mutations result from
either interference of the mutated side chain with substrate
binding at the active site or the introduction of bulky or charged groups
at structurally sensitive internal positions in the mol. Attempts to find
second site revertants of five down mutants, altered either at
the plasmin activation site or near the serine at the active site, only
resulted in same-site revertants, with the original or closely related
amino acid restored.

L98 ANSWER 52 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
AN 1992:38583 HCPLUS
DN 116:38583
TI A comparative study of the promotion of tissue plasminogen activator and
pro-urokinase-induced plasminogen activation by
fragments D and E-2 of fibrin
AU Liu, Jian Ning; Gurewich, Victor
CS Inst. Prevent. Cardiovasc. Dis., New England Deaconess Hosp., Boston, MA,
02215, USA
SO Journal of Clinical Investigation (1991), 88(6), 2012-17
CODEN: JCINAO; ISSN: 0021-9738
DT Journal
LA English
AB Plasmin generation by equimolar concns. of tissue plasminogen activator
(t-PA), pro-urokinase (pro-UK),
and urokinase (UK), and a 2-fold higher concentration of a
plasmin-resistant mutant rpro-UK (Ala-158-
pro-UK) was measured on a microtiter plate reader. The
promoting effects on this reaction of equimolar concns. of fibrinogen,
soluble fibrin (Desafib), CNBr fragment FCB-2 (an analog of fragment D), or
purified fragment E-2 were compared. Plasmin generation by t-PA was
moderately promoted by fibrinogen, substantially promoted by Desafib and
FCB-2, but not at all promoted by fragment E-2. By contrast, plasmin
generation by pro-UK or by Ala-158-pro-
UK was not promoted either by fibrinogen, Desafib, or FCB-2, but
was significantly promoted by any of the fibrin(ogen) preps. Treatment
of fragment E-2 by carboxypeptidase-B (CPB), eliminated its
promotion of pro-UK and Ala-158-pro-

UK-induced plasmin generation. Pretreatment of FCB-2 with plasmin slightly potentiated its promotion of t-PA activity. This effect of plasmin pretreatment of FCB-2 was reversed by CPB treatment. Plasmin pretreatment of FCB-2 did not induce any promotion of activity in pro-UK or Ala-158-pro-UK. The findings show that the intrinsic activity of pro-UK and the activity of t-PA are promoted by different regions of the fibrin(ogen) mol. The latter is stimulated primarily by a determinant in the fragment D region, which is available in intact fibrin. By contrast, plasminogen activation by the intrinsic activity of pro-UK was stimulated exclusively by fragment E-2, which is unavailable in intact fibrin. The findings are believed relevant to fibrinolysis and support the concept that t-PA and pro-UK are complementary, sequential, and synergistic in their actions.

IT 82657-92-9, Pro-urokinase
 RL: BIOL (Biological study)
 (plasminogen activation induction by, fibrinogen degradation products D and E2 effect on)

L98 ANSWER 53 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1991:672471 HCPLUS

DN 115:272471

TI Use of the urokinase-type plasminogen activator gene as a general tool to monitor expression in transgenic animals: study of the tissue-specificity of the murine whey acidic protein (WAP) expression signals

AU Brandazza, Anna; Lee, Eric; Ferrera, Monica; Tillman, Ulrich; Sarmientos, Paolo; Westphal, Heiner

CS Dep. Biotechnol., Farmitalia Carlo Erba, Milan, 20146, Italy

SO Journal of Biotechnology (1991), 20(2), 201-12

CODEN: JBITD4; ISSN: 0168-1656

DT Journal

LA English

AB Urokinase-type plasminogen activator (uPA) is a proteolytic enzyme able to convert the zymogen plasminogen into the strong protease plasmin. The availability of very sensitive tests to measure the enzymic activity of a plasminogen activator renders the corresponding gene an ideal candidate for the detection of promoter activity. In this paper the authors describe the utilization of the human uPA gene as detector of tissue-specificity of the murine whey acidic protein (WAP) expression signals in transgenic mice. The WAP promoter has been previously investigated for the production of foreign proteins in the milk of transgenic animals. In the genetic constructions prepared here, the human uPA cDNA was linked to the promoter region as well as to 3'-end distal sequences of the WAP gene. Five transgenic lines were obtained in which, however, expression levels of human uPA in the milk were still quite low. Surprisingly, 4 of these 5 pos. transgenic mice show a consistent activity of the WAP promoter in brain exts. compared to other tissues.

L98 ANSWER 54 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1991:649567 HCPLUS

DN 115:249567

TI Modification of Shine/Dalgarno sequence and its effect on gene expression

IN Kubo, Miki; Higo, Hirohito

PA Tosoh Corp., Japan

SO Jpn. Kokai Tokkyo Koho, 8 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

PATENT NO.

KIND DATE

APPLICATION NO.

DATE

PI JP 03094686 A2 19910419 JP 1989-233281 19890908 <--
 PRAI JP 1989-233281 19890908 <--

AB The **Shine/Dalgarno** (SD) sequence of a gene or the DNA sequence is modified by substitution to ease/hinder the formation of secondary structure, i.e. to change the free energy required for pairing between 2 bases. By such modification the gene expression, and therefore the protein production, can be regulated in a **recombinant** microbial host. The SD sequence and its surrounding regions of **prourokinase** gene (on plasmid pMUTBS), that formed a secondary structure with a free energy of -10.2 kcal/mol, was modified with 2 synthetic oligonucleotides to increase the free energy to 0 (hindered). Plasmid pMUTRE carrying the modified gene was prepared and used for transformation of **Escherichia coli**. **Prourokinase** produced by the transformants was 4-fold higher than the control.

IT 82657-92-9, **Prourokinase**

RL: PRP (Properties)
 (gene for, expression in **Escherichia coli** of, effect of internal secondary **Shine Dalgarno** structure on)

L98 ANSWER 55 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1991:221530 HCPLUS

DN 114:221530

TI A six-amino acid deletion in basic fibroblast growth factor dissociates its mitogenic activity from its plasminogen activator-inducing capacity

AU Isacchi, Antonella; Statuto, Massimo; Chiesa, Roberta; Bergonzoni, Laura; Rusnati, Marco; Sarmientos, Paolo; Ragnotti, Giovanni; Presta, Marco

CS Sch. Med., Univ. Brescia, Brescia, 25124, Italy

SO Proceedings of the National Academy of Sciences of the United States of America (1991), 88(7), 2628-32

CODEN: PNASA6; ISSN: 0027-8424

DT Journal

LA English

AB A **recombinant** deletion **mutant** of the 155-amino acid form of human basic fibroblast growth factor (bFGF), lacking amino acid residues 27-32 (Lys-Asp-Pro-Lys-Arg-Leu), was expressed in **Escherichia coli** and purified to homogeneity by heparin-Sepharose affinity chromatog. When maintained in the presence of an equimolar concentration of soluble heparin, the bFGF **mutant** (M1-bFGF) is as potent as bFGF in stimulating cell proliferation in normal and transformed fetal bovine aortic endothelial cells, in adult bovine aortic endothelial cells, and in NIH 3T3 fibroblasts. However, under the same exptl. conditions, M1-bFGF is at least 100-fold less efficient than bFGF in stimulating plasminogen activator (PA) production in endothelial cells, as assayed by chromogenic PA assay, SDS/PAGE zymog., and Northern blot anal. of **urokinase**-type PA mRNA. In the presence of heparin, M1-bFGF binds to bFGF plasma membrane receptors present on endothelial cells in a manner indistinguishable from that of bFGF. It also induces the same tyrosine phosphorylation pattern when added to NIH 3T3 cells. Thus, the PA-inducing activity of bFGF may depend upon a functional domain that differs from those involved in the mitogenic activity of the growth factor, and the binding of bFGF to its plasma membrane receptor may not be sufficient to induce **urokinase**-type PA production in endothelial cells.

L98 ANSWER 56 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1991:156602 HCPLUS

DN 114:156602

TI Efficient renaturation and fibrinolytic properties of **prourokinase** and a deletion **mutant** expressed in **Escherichia coli** as inclusion bodies

AU Orsini, Gaetano; Brandazza, Anna; Sarmientos, Paolo; Molinari, Antonio; Lansen, Jacqueline; Cauet, Gilles
 CS Dep. Biotechnol., Farmitalia C, Erba, Milan, I-20146, Italy
 SO European Journal of Biochemistry (1991), 195(3), 691-7
 CODEN: EJBCAI; ISSN: 0014-2956
 DT Journal
 LA English
 AB **Prourokinase** is a plasminogen activator of 411 amino acids which displays clot-lysis activity through a fibrin-dependent mechanism, and which seems to be a promising agent for the treatment of acute myocardial infarction. The preparation of **recombinant prourokinase** in bacteria has been hampered by its insolubility and by difficulty in refolding the polypeptide chain. In this paper the authors describe the renaturation process of 2 recombinant proteins expressed in *E. coli* as inclusion bodies: **prourokinase** and a deletion derivative (Δ 125- **prourokinase**) in which 125 amino acids of the N-terminal region have been removed. Deletion of this sequence brings higher refolding yields and faster kinetics (first-order rate constant of renaturation of 0.57/h for Δ 125- **prourokinase** and 0.25/h for **prourokinase**). The process involves sequential steps of denaturation, reduction and controlled refolding of the polypeptide chain. When applied to pure, non-glycosylated and active **prourokinase**, it gives a refolding yield of about 80%, demonstrating the efficiency of the renaturation procedure. Lower yields (15 and 30%, resp., for **prourokinase** and Δ 125- **prourokinase**) were obtained when the same refolding protocol was applied to inclusion bodies from bacteria. After purification to homogeneity (as shown by HPLC and SDS/PAGE) specific activities were 160000 and 250000 IU/mg protein, resp., for **prourokinase** and Δ 125- **prourokinase**. As with **prourokinase**, the deletion mutant Δ 125- **prourokinase** displays a zymogenic nature, being activated by plasmin to the active two-chain form; however, this mutant is approx. 4-fold more resistant than **prourokinase** to plasmin activation, and consequently shows a different fibrinolytic profile.
 IT 82657-92-9, **Prourokinase**
 RL: BIOL (Biological study)
 (efficient renaturation and fibrinolytic property of a deletion mutant expressed in *Escherichia coli* as inclusion bodies and, structure in relation to)
 L98 ANSWER 57 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1991:137351 HCPLUS
 DN 114:137351
 TI Optimizing the promoter and ribosome binding sequence for expression of human **single chain urokinase-like plasminogen activator** in *Escherichia coli* and stabilization of the product by avoiding heat shock response
 AU Surek, Baerbel; Wilhelm, Martin; Hillen, Wolfgang
 CS Inst. Mikrobiol. Biochem., Friedrich-Alexander-Univ., Erlangen, D-8520, Germany
 SO Applied Microbiology and Biotechnology (1991), 34(4), 488-94
 CODEN: AMBIDG; ISSN: 0175-7598
 DT Journal
 LA English
 AB The expression of recombinant **single-chain urokinase-like plasminogen activator** (rscuPA) in *Escherichia coli* was optimized by fusing the **puk** gene to different promoters and ribosome binding sequences.. Comparison of the tac, trp and λ PL promoters showed that expression was maximal under tac control. Variation in the ribosome binding sequence and its distance to the AUG start codon yielded a further slight improvement of expression. The largest increase in rscuPA expression was achieved by

variations in the host strain and growth conditions. In *E. coli* DG75 grown at 37° maximal expression was achieved 30 min after induction and decreased gradually until 240 min after induction. Growth at 30° yielded maximal expression 60 min after induction and resulted in reduced activity at longer times. Western blot anal. of the products showed that degradation of rscuPA was much larger at 37° than at 30°. Using *E. coli* CAG630 carrying the htpR mutation, which avoids heat shock response, for expression of rscuPA eliminated the instability of the product at both temps. Expression in this strain was even more efficient than in *E. coli* JM101 carrying the lon mutation. It is concluded that induction of the general heat-shock response in *E. coli* must be avoided to obtain stabilization of rscuPA. This drastically improves the overall yield of rscuPA from recombinant *E. coli* strains.

L98 ANSWER 58 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1991:117685 HCAPLUS
 DN 114:117685
 TI Stabilization of freeze-dried prourokinase
 IN Morimoto, Kazuo; Narita, Shusaku; Nishikawa, Masaru; Takechi, Kazuo
 PA Green Cross Corp., Japan
 SO Eur. Pat. Appl., 6 pp.
 CODEN: EPXXDW
 DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 391400	A2	19901010	EP 1990-106492	19900405 <--
	EP 391400	A3	19901114		
	R: BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
	JP 02268681	A2	19901102	JP 1989-86932	19890407 <--
	CA 2014009	AA	19901007	CA 1990-2014009	19900406 <--
PRAI	JP 1989-86932	A	19890407 <--		
AB	Prourokinase for use in pharmaceuticals is stabilized as a dry powder by incorporating a polar amino acid (acidic or basic) or their salts into the prourokinase solution before freeze drying. The amino acid is added at 5-30 mg per 10,000-250,000 IU prourokinase. Samples of prourokinase 25,000 IU in phosphate buffer were freeze-dried in the presence of 6.4 mg/mL of aspartate, glutamate, Na glutamate, arginine, lysine, or histidine. Recovery of activity immediately after lyophilization was 96% in all cases (94% in a control with no addns.). After 3 mo at 50° the test samples retained 56-63% of activity whereas the control sample (no addns.) had retained only 2% of its activity. This stabilization was also shown to be a function of the concentration of the stabilizer.				
IT	82657-92-9, Prourokinase RL: BIOL (Biological study) (freeze-dried, stabilization of, polar amino acids for)				
IT	56-87-1, Lysine, biological studies 71-00-1, Histidine, biological studies RL: BIOL (Biological study) (prourokinase stabilized by, after freeze-drying)				

L98 ANSWER 59 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1991:69055 HCAPLUS
 DN 114:69055
 TI Pharmaceutical composition comprising a plasminogen activator and hirudin
 IN Heim, Jutta; Agnelli, Giancarlo; Czendlik, Czeslaw
 PA Ciba-Geigy A.-G., Switz.; UCP Gen-Pharma A.-G.
 SO Eur. Pat. Appl., 33 pp.
 CODEN: EPXXDW

DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 365468	A1	19900425	EP 1989-810676	19890912 <--
	EP 365468	B1	19940216		
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	AT 101524	E	19940315	AT 1989-810676	19890912 <--
	AU 8941352	A1	19900329	AU 1989-41352	19890913 <--
	AU 628854	B2	19920924		
	US 5126134	A	19920630	US 1989-408836	19890918 <--
	CA 1336493	A1	19950801	CA 1989-611903	19890919 <--
	DK 8904639	A	19900322	DK 1989-4639	19890920 <--
	JP 02121934	A2	19900509	JP 1989-242356	19890920 <--
	ZA 8907173	A	19900926	ZA 1989-7173	19890920 <--
	IL 91706	A1	19970713	IL 1989-91706	19890920 <--
	KR 149001	B1	19981015	KR 1989-13492	19890920 <--
PRAI	GB 1988-22147	A	19880921	<--	
	EP 1989-810676	A	19890912	<--	
AB	Pharmaceutical compns. containing a plasminogen activator and a hirudin can be used for prophylaxis and therapy of thrombosis or diseases caused by thrombosis. The dissoln. of thrombi is accelerated significantly and the risk of reocclusion is considerably reduced when using this combination rather than a plasminogen activator alone. Lysis of thrombi were observed with a rabbit jugular vein thrombosis model; i.v. administration of tissue plasminogen activator (tPA) alone, of tPA and heparin, and of tPA and hirudin produced 37-44, 34, and 52% clot lysis, resp. Addnl., the presence of hirudin decreased thrombin accretion by approx. 50% relative to tPA alone or to tPA and heparin. Expression vectors for manufacture of hirudin mutants in <i>Escherichia coli</i> and yeast were prepared				
IT	121449-21-6	121449-35-2			
	RL: BIOL (Biological study)				
	(as thrombolytic, improved rate of clot lysis and inhibition of thrombus accretion with)				
IT	131790-70-0	131790-74-4	131790-75-5		
	131877-61-7				
	RL: BIOL (Biological study)				
	(as thrombolytic, increased rate of clot lysis and decreased clot accretion with)				
L98	ANSWER 60 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN				
AN	1991:18956 HCPLUS				
DN	114:18956				
TI	Manufacture of prourokinase with <i>Escherichia coli</i>				
IN	Brandazza, Anna; Sarmientos, Paolo; Orsini, Gaetano				
PA	Farmitalia Carlo Erba S.r.l., Italy				
SO	PCT Int. Appl., 38 pp.				
	CODEN: PIXXD2				
DT	Patent				
LA	English				
FAN.CNT 1					
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9004023	A1	19900419	WO 1989-EP1168	19891006 <--
	W: AT, DK, FI, HU, JP, KR, NO, SU, US				
	RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
	EP 365894	A1	19900502	EP 1989-118586	19891006 <--
	R: ES, GR				
	EP 407490	A1	19910116	EP 1989-911367	19891006 <--
	R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				

HU 55443	A2	19910528	HU 1989-6054	19891006 <--
HU 209149	B	19940328		
JP 03502526	T2	19910613	JP 1989-510599	19891006 <--
CA 2000408	AA	19900411	CA 1989-2000408	19891010 <--
CN 1042181	A	19900516	CN 1989-108587	19891010 <--
AU 8943823	A1	19910502	AU 1989-43823	19891026 <--
AU 624869	B2	19920625		
NO 9002564	A	19900809	NO 1990-2564	19900608 <--
DK 9001410	A	19900813	DK 1990-1410	19900608 <--
US 5866358	A	19990202	US 1990-536556	19900711 <--

PRAI GB 1988-23833 A 19881011 <--
 WO 1989-EP1168 W 19891006 <--

AB Human prourokinase is manufactured with recombinant *E. coli*. The prourokinase cDNA is expressed in *E. coli* B under the control of the *Ptrp* promoter and the Shine-Dalgarno sequence MS-2.

L98 ANSWER 61 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 1990:545081 HCAPLUS

DN 113:145081

TI Characterization of thrombin- and plasmin-resistant mutants of recombinant human single chain urokinase-type plasminogen activator

AU Eguchi, Yutaka; Sakata, Yoichi; Matsuda, Michio; Osada, Hiroshi; Numao, Naganori; Ohmori, Muneki; Kondo, Kiyoshi

CS Inst. Hematol., Jichi Med. Sch., Tochigi, 329-04, Japan

SO Journal of Biochemistry (Tokyo, Japan) (1990), 108(1), 72-9
 CODEN: JOBIAO; ISSN: 0021-924X

DT Journal

LA English

AB Recombinant human single-chain urokinase-type plasminogen activator (Scu-PA) (SM0: wild type) and its variants resistant to plasmin and/or thrombin (SM1:Lys135 → Gln; SM3:Phe157 → Asp; and SM4:Lys135 → Gln and Phe157 → Asp) have been constructed by site-directed mutagenesis with the aim of producing more efficient thrombolytic agents. The recombinant variant scu-PAs expressed in *Escherichia coli* were characterized. They appeared to have structural integrity because their heat stabilities, immunol. reactivities, and CD spectra were essentially identical to each other and to those of native scu-PA (nscu-PA). In the presence of thrombin, SM3 and SM4 caused efficient clot lysis in all of the assays used, compared with SM0, SM1, and nscu-PA. In the absence of thrombin, when measured by a fibrin plate method in a purified system, SM3 and SM4 had lower sp. activities than SM0, SM1, and nscu-PA, since their catalytic consts. for conversion to the 2-chain form by plasmin were lower. SM4 lysed clots as efficiently as SM0 in plasma by retaining the single-chain form, whereas SM0 was partly converted to the 2-chain form.

L98 ANSWER 62 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1990:491463 HCAPLUS

DN 113:91463

TI Low molecular weight derivatives of prourokinase as thrombolytics

IN Brandazza, Anna; Lansen, Jacqueline; Mazue, Guy; Sarmientos, Paolo

PA Farmitalia Carlo Erba S.r.l., Italy

SO Eur. Pat. Appl., 16 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO.

KIND

DATE

APPLICATION NO.

DATE

PI	EP 338409	A1	19891025	EP 1989-106549	19890413 <--
	EP 338409	B1	19950201		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, NL, SE				
	WO 8910402	A1	19891102	WO 1989-EP394	19890413 <--
	W: AU, DK, FI, HU, JP, KR, NO, SU, US				
	AU 8934149	A1	19891124	AU 1989-34149	19890413 <--
	AU 617459	B2	19911128		
	HU 52558	A2	19900728	HU 1989-2527	19890413 <--
	JP 03500125	T2	19910117	JP 1989-504110	19890413 <--
	CN 1037360	A	19891122	CN 1989-102351	19890418 <--
	ZA 8902829	A	19900131	ZA 1989-2829	19890418 <--
	IL 90011	A1	19941021	IL 1989-90011	19890418 <--
	NO 8905078	A	19900216	NO 1989-5078	19891215 <--
	DK 8906416	A	19891218	DK 1989-6416	19891218 <--

PRAI	GB 1988-9093	A	19880418	<--
	WO 1989-EP394	A	19890413	<--

AB Low mol.-weight derivs. of human prourokinase are provided which comprise at least the sequence from amino acid 163 to amino acid 411 of mature prourokinase in single chain configuration. In preferred cases, the sequences making up the kringle domain, the receptor binding domain or cell binding domain are missing. The derivs. show thrombolytic activity. Modified genes encoding for these derivs. were constructed by site-specific mutagenesis. Using traditional expression plasmids, the mutated genes were expressed in recombinant *Escherichia coli* strains. The amidolytic behavior of the new derivs. Δ 125, Δ 140 and Δ 150 Δ = deleted amino acid sequences indicated that any mol. which keeps at least 261 amino acids of prourokinase in a single chain configuration could be fully amidolytically active upon plasmin activation by conversion in the two-chain configuration. Heparin, in concns. which can be reached in plasma during therapeutic anticoagulation (0.01-1 IU/mL), concomitantly to thrombolytic therapy, stimulated the conversion of Δ 125 in its two-chain configuration, by plasmin. This effect was also obtained using wild-type prourokinase, but to a much lesser extent. Almost complete lysis of 125I-labeled human fibrin clots were obtained with the derivs., without fibrinolysis, which was not the case for two-chain urokinase. This effect was maintained after \geq 24 h incubation of the derivs. in plasma prior to the addition of the 125I-labeled human fibrin clot. On the contrary, two-chain urokinase clot lysis activity disappeared completely after long term pre-incubation in human plasma. Long half-life in the blood was observed after single i.v. injection of Δ 125.

IT 82657-92-9DP, Prourokinase, low-mol. weight derivs.

RL: SPN (Synthetic preparation); PREP (Preparation)
(preparation of, as thrombolytic agents)

L98 ANSWER 63 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
AN 1990:401558 HCPLUS

DN 113:1558

TI Recombinant human single-chain urokinase-type plasminogen activator mutant produced by site-specific mutagenesis of lysine 158 to histidine 158

IN Collen, Desire Jose; Nelles, Lucien Georges Raymond; Lijnen, Henri Roger; De Cock, Frans; Van Hoef, Berthe; Stassen, Jean Marie

PA Leuven Research and Development V.Z.W., Belg.

SO Eur. Pat. Appl., 15 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	-----	-----	-----	-----

PI EP 336508 A1 19891011 EP 1989-200825 19890330 <--
 R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE
 PRAI US 1988-178205 A 19880406 <--
 AB Single chain human [His158] urokinase has kinetic and inactivation properties and in vivo behavior in animal test systems that make it suitable for use in the treatment of thromboembolism. A cDNA encoding this protein was prepared by standard site-directed mutagenesis methods and the protein manufactured by expression of the gene in CHO cells. The purified protein was converted to the two-chain form by plasmin but not by thrombin. Activation of plasminogen by this protein was as effective as that of unmodified urokinase. Tests of stimulation of thrombolysis in vivo (rabbit jugular vein model) showed a linear dose-response curve in the range 0.5-2.0 mg urokinase/kg.

L98 ANSWER 64 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1990:133816 HCPLUS
 DN 112:133816
 TI Recombinant manufacture of thrombin-resistant prourokinase derivatives with amino acid substitutions at position 156
 IN Koerwer, Wolfgang; Kurfuerst, Manfred; Baldinger, Verena; Doerper, Thomas
 PA BASF A.-G., Fed. Rep. Ger.
 SO Eur. Pat. Appl., 20 pp.
 CODEN: EPXXDW
 DT Patent
 LA German
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 312942	A2	19890426	EP 1988-117188	19881015 <--
	EP 312942	A3	19900502		
	R: AT, BE, CH, DE, ES, FR, GB, IT, LI, NL				
	DE 3735916	A1	19890503	DE 1987-3735916	19871023 <--
	DK 8805875	A	19890614	DK 1988-5875	19881021 <--
	JP 01160482	A2	19890623	JP 1988-266255	19881024 <--

PRAI DE 1987-3735916 A 19871023 <--
 AB Prourokinases with amino acid substitutions at position 156 which are resistant to thrombin inhibition are prepared by in vitro mutagenesis of a cDNA and manufactured by expression in *Escherichia coli*. These derivs. have properties better suited for use as pharmaceuticals. Prourokinases with arginine-156 replaced by glutamic acid or leucine were prepared by oligonucleotide-directed site-specific mutagenesis and manufactured by expression in *E. coli*. The inhibition of amidolytic activity by thrombin was compared. Although the normal prourokinase is reduced to 36% of control activity by incubation with 10 units of thrombin at 37° for 30 min, the substituted derivs. retained 100% activity after 1 h incubation under the same conditions.

IT 125959-05-9 125959-06-0 125959-07-1
 RL: PRP (Properties); BIOL (Biological study)
 (nucleotide sequence and expression in *Escherichia coli* of)
 IT 82657-92-9D, Prourokinase, derivs.
 RL: PRP (Properties)
 (thrombin-resistant of, cloning and expression in *Escherichia coli* of cDNA for)

L98 ANSWER 65 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1990:133815 HCPLUS
 DN 112:133815
 TI Polypeptides with a prourokinase activity, their production and use

IN Koerwer, Wolfgang; Kurfuerst, Manfred; Baldinger, Verena; Doerper, Thomas;
Schwarz, Margarete

PA BASF A.-G., Fed. Rep. Ger.

SO Eur. Pat. Appl., 21 pp.

CODEN: EPXXDW

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 312941	A2	19890426	EP 1988-117186	19881015 <--
	EP 312941	A3	19900516		
	R: AT, BE, CH, DE, ES, FR, GB, IT, LI, NL				
	DE 3735917	A1	19890503	DE 1987-3735917	19871023 <--
	DK 8805876	A	19890614	DK 1988-5876	19881021 <--
	JP 01160481	A2	19890623	JP 1988-266254	19881024 <--

PRAI DE 1987-3735917 A 19871023 <--

AB Prourokinases with amino acid substitutions at position 156 and N-terminal extensions resistant to thrombin inhibition and with prolonged half-lives in vivo are prepared by in vitro mutagenesis of a cDNA and manufactured by expression in *Escherichia coli*. These derivs. have properties better suited for use as pharmaceuticals. Polypeptides with an addnl. N-terminal Met and Arg156 (I) or Lys156 (II) were injected into male Sprague-Dawley rats at 1 mg/kg. The half-life of the naturally-occurring form of the protein was 3 min whereas for I it was 10 min and \geq 10 min for II.

IT 125959-08-2 125959-09-3

RL: PRP (Properties); BIOL (Biological study)
(nucleotide sequence and expression in *Escherichia coli* of)

IT 82657-92-9D, Prourokinase, derivs.

RL: PRP (Properties)
(thrombin resistant, long serum half-life, cDNA for, cloning in *Escherichia coli* of)

L98 ANSWER 66 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1990:113619 HCPLUS

DN 112:113619

TI Human prourokinase variants and their recombinant manufacture with *Escherichia coli*

IN Tagawa, Michito; Kobayashi, Yoichi; Yamada, Masayuki; Wada, Masakatsu; Mukohara, Yukio; Fushimi, Takaomi; Hachiman, Hideo; Omori, Muneki; Yokoyama, Midori; Et, Al.

PA Sagami Chemical Research Center, Japan; Central Glass Co., Ltd.; Hodogaya Chemical Co., Ltd.

SO Jpn. Kokai Tokkyo Koho, 15 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 01187087	A2	19890726	JP 1988-9369	19880119 <--
PRAI	JP 1988-9369		19880119 <--		

AB Human prourokinase mutants with amino acid substitutions at Lys-135 or Phe-157, or at both, are manufactured by expression of the mutated genes in *Escherichia coli* from plasmids from specified regulatory sequences such as the trp promoter/operator, or tac or lac or lacUV5 promoter/operator in combination with an upstream lac repressor such as lacIq. Expression plasmids pUKO2pm4, pRQPSDpm4, and pRQCSDpm4 encoding [Gln-135, Asp-157] prourokinase and expression plasmid pUKO1pm1 encoding [Gln-135] prourokinase were constructed and used for gene expression with

yields 2-3.5-fold higher than the prior art.

IT 82657-92-9

RL: PRP (Properties)
(amino acid substituted, gene for, expression in *Escherichia coli* of)

L98 ANSWER 67 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1990:94567 HCAPLUS

DN 112:94567

TI A recombinant pro-urokinase derived mutant missing the growth factor-like domain does not bind to its receptor

AU Robbiati, F.; Nolli, M. L.; Soffientini, A.; Sarubbi, E.; Stoppelli, M. P.; Cassani, G.; Parenti, F.; Blasi, F.

CS Lepetit Res. Cent., Merrel Dow Res. Inst., Gerenzano, 21040, Italy

SO Fibrinolysis (1990), 4(1), 53-60

CODEN: FBRIE7; ISSN: 0268-9499

DT Journal

LA English

AB Prourokinase (pro-u-PA) is the single chain precursor of the urokinase-type plasminogen activator u-PA. Both u-PA and pro-u-PA bind to an extracellular receptor present on the membrane of several cell types of both malignant and normal origin, including endothelial cells. Competition expts. with u-PA fragments and synthetic peptides have suggested that the growth factor-like domain (GFD) of u-PA is involved in receptor binding. To prove the direct involvement of the GFD in receptor binding, a mutant u-PA gene missing the GFD was constructed and expressed. Mutant 9Y45 has u-PA introns C and D fused together, thus deleting exon IV that codes for the GFD (amino acids 9-45). The product of the mutant gene is a single chain, pro-u-PA derived protein with an apparent Mr .apprx.43 kDa that, upon conversion to the 2 chain form, acquires full enzymic activity, suggesting that the mol. has preserved most of its original structure. Mutant 9Y45 is recognized by an anti-human u-PA serum, by a monoclonal antibody directed against the kringle domain but not by a monoclonal raised against the GFD. N-terminal and C-terminal amino acid sequencing of the purified protein confirms that: (a) the GFD is absent; (b) the difference in MW is not due to a truncated protein; (c) the missing amino acids 9-45 are substituted by a novel tyrosine joining the last amino acid in exon III to the first amino acid of exon V. Mutant 9Y45 does not bind to the u-PA receptor as shown by its inability to compete with ¹²⁵I-labeled DFP-treated u-PA for binding to the u-PA receptor of human U937 cells, even at a concentration 1000-fold higher

than

that of control pro-u-PA. Thus, the most important receptor binding determinants of pro-u-PA (and u-PA) reside in the GFD.

IT 82657-92-9, Prourokinase

RL: PRP (Properties)
(growth factor-like domain of, urokinase-type plasminogen activator-receptor binding requirement for)

L98 ANSWER 68 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1989:513738 HCAPLUS

DN 111:113738

TI *Escherichia coli* with lon gene suppression and its use for efficient manufacture of recombinant proteins

IN Tagawa, Naoto; Yamada, Masayuki; Kakiya, Hitoshi; Numao, Osanori; Yura, Takashi

PA Sagami Chemical Research Center, Japan; Central Glass Co., Ltd.; Hodogaya Chemical Co., Ltd.; Nippon Soda Co., Ltd.; Nissan Chemical Industries, Ltd.

SO Jpn. Kokai Tokkyo Koho, 6 pp.
CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 63129981	A2	19880602	JP 1986-274105	19861119 <--
	JP 07077555	B4	19950823		
PRAI	JP 1986-274105		19861119 <--		
AB	<p><i>Escherichia coli</i> deficient in tRNA suppressor activity (sup-) and lon gene expression (due to mutation of the htpR gene) in the temperature range optimal for cell growth or gene expression for heterologous proteins is used for manufacturing the proteins. <i>E. coli</i> strain KY1436 transformed with plasmid pHA20 encoding plasminogen activator was cultivated in L medium (5 mL) at 30° with agitation until the cell concentration reached OD600 0.4, followed by shifting to 37° and cultivating an addnl. 6 h. It was recovered from the cells and identified by SDS-PAGE.</p>				
IT	<p>82657-92-9P, Prourokinase RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation) (manufacture of, with <i>Escherichia coli</i> with inactivated lon gene and tRNA suppressor)</p>				

L98 ANSWER 69 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1989:434671 HCPLUS

DN 111:34671

TI Single-stranded hybrid plasminogen activators, their recombinant manufacture, and pharmaceutical compositions containing them

PA Ciba-Geigy A.-G., Switz.

SO Jpn. Kokai Tokkyo Koho, 72 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 63160581	A2	19880704	JP 1987-306016	19871204 <--
	JP 2641875	B2	19970820		
	FI 8705324	A	19880606	FI 1987-5324	19871202 <--
	FI 100106	B1	19970930		
	EP 277313	A1	19880810	EP 1987-117892	19871203 <--
	EP 277313	B1	19970122		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	IL 84700	A1	19921115	IL 1987-84700	19871203 <--
	AT 148167	E	19970215	AT 1987-117892	19871203 <--
	ES 2095825	T3	19970301	ES 1987-117892	19871203 <--
	DK 8706381	A	19880606	DK 1987-6381	19871204 <--
	DK 175483	B1	20041108		
	NO 8705069	A	19880606	NO 1987-5069	19871204 <--
	NO 177603	B	19950710		
	NO 177603	C	19951018		
	AU 8782091	A1	19880616	AU 1987-82091	19871204 <--
	AU 621281	B2	19920312		
	JP 09117292	A2	19970506	JP 1996-260999	19871204 <--
	US 5242819	A	19930907	US 1991-808936	19911213 <--
	US 5580559	A	19961203	US 1994-311848	19940923 <--
PRAI	GB 1986-29153	A	19861205	<--	
	GB 1987-1160	A	19870120	<--	
	GB 1987-9656	A	19870423	<--	
	GB 1987-15890	A	19870706	<--	

US 1987-125039	B2	19871123	<--
JP 1987-306016	A3	19871204	<--
US 1989-361015	B1	19890602	<--
US 1991-808936	A3	19911213	<--
US 1993-49469	B1	19930419	<--

AB Recombinant single-stranded human hybrid plasminogen activators (PA) comprised of part or all of urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA), or muteins thereof, are prepared. The novel hybrid PAs have improved affinity for fibrin and stability in vivo. Plasmid pJDB207/PH05-I-MOUK2TPAB comprising the PH05 gene promoter, the invertase gene signal sequence, and a chimeric gene encoding the kringle 2 domain of uPA fused to the B-chain of tPA [i.e., uPA(1-44)-tPA(176-527)] was constructed. This hybrid PA, i.e. UK2TPAB, was recovered from the cell homogenate of transformed *Saccharomyces cerevisiae* and purified. with monoclonal antibodies and chromatog. The thrombolytic activity of UK2TPAB was similar to t-PA, but its half-life in peripheral blood of rabbits was 10 times greater (20 vs. 2 min). Parenteral preparation containing UK2TPAB was prepared

IT 121449-18-1 121449-19-2 121449-20-5
 121449-21-6 121449-22-7 121449-23-8
 121449-24-9 121449-25-0 121449-26-1
 121449-27-2 121449-28-3 121449-29-4
 121449-30-7 121449-33-0 121449-34-1
 121449-35-2

RL: PRP (Properties)
 (amino acid sequence of and cloning and expression in eukaryotes of cDNA for)

L98 ANSWER 70 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1989:2184 HCPLUS

DN 110:2184

TI Recombinant urokinase-tissue plasminogen activator fusion proteins

IN Bollen, Alex Joseph; Gheysen, Dirk; Jacobs, Paul; Pierard, Laurent; Collen, Desire J.

PA Smith Kline-RIT S. A., Belg.; Leuven Research and Development VZW

SO PCT Int. Appl., 51 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 8804690	A1	19880630	WO 1987-BE18	19871211 <--
	W: AU, DK, JP				
	ZA 8709286	A	19881026	ZA 1987-9286	19871210 <--
	AU 8811035	A1	19880715	AU 1988-11035	19871211 <--
	EP 275856	A1	19880727	EP 1987-870176	19871211 <--
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 02501106	T2	19900419	JP 1987-506364	19871211 <--
	DK 8804590	A	19880816	DK 1988-4590	19880816 <--

PRAI US 1986-942127 A 19861216 <--
 WO 1987-BE18 A 19871211 <--

AB Plasmids encoding fusion proteins comprising tissue-type plasminogen activator chain A-derived peptide and urokinase chain B-derived peptide are constructed and expressed in mammalian cells. Plasmid pULB9151, containing DNA encoding a 262 amino acid N-terminal peptide from tissue plasminogen activator fused to DNA encoding the C-terminal part of urokinase (from residue 139 to the end), was constructed. Recombinant fusion protein produced by COS1 or R1610 cells transfected with this DNA had a specific activity of 73,000 units/mg.

L98 ANSWER 71 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1988:628605 HCPLUS
 DN 109:228605
 TI Yeast secretion of mammalian proteins: a comparison of prourokinase, prochymosin and alpha-1-antitrypsin
 AU Moir, Donald T.
 CS Collab. Res., Inc., Bedford, MA, 01730, USA
 SO World Biotech Rep. (1987), Volume 1, Issue Pt. 3, 39-45
 Publisher: Online Publ., London, UK.
 CODEN: 56IUA3
 DT Conference
 LA English
 AB Mammalian proteins secreted from bakers' yeast are modified post-translationally in two important ways: disulfide bonds form properly and the correct asparagines are glycosylated. The secretion signal, host strain, mode of maintenance in the cell, and the particular mammalian gene all influence the efficiency of secretion from yeast. Calf prochymosin and human prourokinase secreted from yeast, in contrast to those proteins produced intracellularly in *Escherichia coli* or yeast, are properly folded and exhibit full specific activity. Human alpha-1-anti-trypsin secreted from yeast is glycosylated at all three asparagines, and yeast mutants alter the pattern of the carbohydrate applied.
 IT 82657-92-9P, Prourokinase
 RL: PREP (Preparation)
 (manufacture of human, by recombinant yeast)

L98 ANSWER 72 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1988:523794 HCPLUS
 DN 109:123794
 TI Human prourokinase mutants, method for producing the same, DNA sequences encoding them, plasmids containing the same, and transformants containing them
 IN Kasai, Shunji; Hiramatsu, Ryuji; Uno, Shusei; Nagai, Masanori; Arimura, Hirofumi
 PA Green Cross Corp., Japan
 SO Eur. Pat. Appl., 41 pp.
 CODEN: EPXXDW
 DT Patent
 LA English
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 253241	A1	19880120	EP 1987-109628	19870703 <--
	R: BE, CH, DE, ES, FR, GB, LI, NL, SE				
	JP 63146789	A2	19880618	JP 1987-36495	19870218 <--
	JP 07061266	B4	19950705		
	US 5098840	A	19920324	US 1990-525011	19900518 <--
PRAI	JP 1986-156936	A	19860703	<--	
	JP 1987-36495	A	19870218	<--	
	US 1987-70003	B2	19870706	<--	
	JP 1989-126433	A	19890518	<--	
	JP 1989-126434	A	19890518	<--	
	US 1989-433938	B2	19891109	<--	
	JP 1990-42020	A	19900222	<--	

AB A human prourokinase mutant (I) having longer half-life in blood and less bleeding side-effects is manufactured using genetic engineering techniques. Some or all of the epidermal growth factor (EGF) domain has been deleted or replaced by one or more different amino acid residues. The synthetic oligonucleotide encoding the mutant I lacking the Asn10-Asp45 region was prepared and used in constructing the expression plasmid pSV-UK11. CHO-K1 cells transformed with pSV-UK11

produced I having a half-life in blood of 6.25 min and similar activity to the natural human urine **urokinase**.

IT 116284-13-0, Deoxyribonucleic acid (human clone pUK4/pUK18 (1-9) - (43-411) **prurokinase**-specifying) 116284-14-1, Deoxyribonucleic acid (human clone pUK4/pUK18 (1-9) - (46-411) - **prurokinase**-specifying) 116284-15-2, Deoxyribonucleic acid (human clone pUK4/pUK18 (1-9) - (50-411) - **prurokinase**-specifying)
 RL: PRP (Properties)
 (expression in CHO cells and nucleotide sequence of)

L98 ANSWER 73 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN

AN 1988:433212 HCPLUS

DN 109:33212

TI Construction of **Escherichia coli** expression vectors encoding hybrid tissue plasminogen activator-**urokinase** proteins, and preparation of said proteins

IN Tagawa, Michito; Wada, Masakatsu; Yamada, Masayuki; Yokoyama, Midori; Numao, Naganori

PA Sagami Chemical Research Center, Japan; Central Glass Co., Ltd.; Hodogaya Chemical Co., Ltd.; Nippon Soda Co., Ltd.; Nissan Chemical Industries, Ltd.; Toyo Soda Mfg. Co., Ltd.

SO Eur. Pat. Appl., 65 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 231883	A1	19870812	EP 1987-101209	19870129 <--
	EP 231883	B1	19920902		
	R: BE, CH, DE, ES, FR, GB, IT, LI, NL, SE				
	ES 2043610	T3	19940101	ES 1987-101209	19870129 <--
	DK 8700509	A	19870801	DK 1987-509	19870130 <--
	DK 175604	B1	20041220		
	WO 8704720	A1	19870813	WO 1987-JP65	19870130 <--
	W: SU				
	JP 62272975	A2	19871127	JP 1987-18626	19870130 <--
	JP 2589687	B2	19970312		
	SU 1732814	A3	19920507	SU 1987-4028997	19870130 <--
	US 5204255	A	19930420	US 1991-726129	19910703 <--
PRAI	JP 1986-17734	A	19860131 <--		
	US 1987-7865	B1	19870128 <--		

AB Hybrid proteins containing N-terminal fibrin binding domain(s) from tissue plasminogen activator (tPA) and a C-terminal **prurokinase** (PK) polypeptide having plasminogen activator activity are produced with **E. coli** transformed with expression vectors encoding these proteins. Plasmid pH403, encoding a hybrid protein consisting of residues 161-219 of tPA at the N-terminal and residues 150-411 of PK in which Phe-157 is changed to aspartic acid (to inhibit proteolytic degradation) at the C-terminal, was constructed. Expression of the protein was under the control of the tac promoter and the C230 Shine-Dalgarno sequence. This hybrid protein, expressed in **E. coli**, had the same affinity for a fibrin affinity column as did tPA; the same enzymic activity as PK, i.e. a $K_m = 2.0 + 10^{-4}$ mol/L (using synthetic substrate S2288); and an increased resistance to proteolytic inactivation by plasmin and thrombin.

IT 115283-71-1 115283-72-2 115283-73-3
 115283-75-5 115283-76-6 115283-77-7
 115283-78-8 115283-79-9 115283-80-2
 115283-81-3

RL: PRP (Properties); BIOL (Biological study)
 (amino acid sequence of and cloning and expression in

IT **Escherichia coli** of cDNA for)
 IT 115283-74-4
 RL: PRP (Properties)
 (amino acid sequence of and cloning and expression in
Escherichia coli of cDNA of)
 IT 82657-92-9D, Prourokinase, fusion products
 RL: PRP (Properties)
 (enzymic region of, with fibrin binding region of tissue-type
 plasminogen activator, manufacture in **Escherichia coli**
 of)
 IT 115283-41-5 115283-42-6 115283-43-7
 115283-44-8 115283-45-9 115283-46-0
 115283-47-1 115283-48-2 115283-49-3
 115283-50-6 115283-51-7
 RL: PRP (Properties); BIOL (Biological study)
 (nucleotide sequence and cloning and expression in **Escherichia coli** of)
 L98 ANSWER 74 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1988:162640 HCPLUS
 DN 108:162640
 TI Enhanced expression of human pro-urokinase cDNA in
Escherichia coli
 AU Hibino, Yasuo; Miyake, Toshio; Kobayashi, Yohichi; Ohmori, Muneki; Miki, Tetsuzo; Matsumoto, Reiko; Numao, Naganori; Kondo, Kiyosi
 CS Sagami Chem. Res. Cent., Kanagawa, 229, Japan
 SO Agricultural and Biological Chemistry (1988), 52(2), 329-36
 CODEN: ABCHA6; ISSN: 0002-1369
 DT Journal
 LA English
 AB Human pro-urokinase cDNA was isolated from the cDNA library constructed from human kidney mRNA using the dC/dG homopolymer tailing method and Okayama-Berg method with pBR322 as a vector. A mature polypeptide starting with Ser was produced in **E. coli** under the control of the tac promoter and the Shine-Dalgarno sequence of the catechol 2,3-oxygenase gene derived from *Pseudomonas putida*. By replacing the sequence coding for N-terminal eight amino acids of pro-urokinase with the synthetic DNA oligomer, the bacterial pro-urokinase had a mol. weight of 47,000 daltons and accounted for 15% of the insol. fraction of **E. coli** proteins in induced cells. Its biol. activity was restored by renaturing the bacterial product. The activity of bacterial pro-urokinase was 450 IU/mL culture.
 IT 82657-92-9, Pro-urokinase
 RL: PRP (Properties)
 (cDNA for, of human, cloning and expression of)
 L98 ANSWER 75 OF 75 HCPLUS COPYRIGHT 2005 ACS on STN
 AN 1985:434019 HCPLUS
 DN 103:34019
 TI Characterization of the high-affinity interaction between human plasminogen and pro-urokinase
 AU Lijnen, H. Roger; Collen, Desire
 CS Cent. Thromb. Vasc. Res., Univ. Leuven, Louvain, Belg.
 SO European Journal of Biochemistry (1985), 150(1), 141-4
 CODEN: EJBCAI; ISSN: 0014-2956
 DT Journal
 LA English
 AB Activation of human plasminogen (I), a modified plasminogen with N-terminal lysine, valine, or methionine (II), and low-mol.-weight plasminogen [lacking lysine-binding sites (LBSSs)] (III) by prourokinase (pro-UK), obtained from a human lung adenocarcinoma cell line (Calu-3, ATCC), obeys Michaelis-Menten

kinetics. Activation occurs with a comparable affinity (K_m, 0.40-0.77 μM), whereas the catalytic rate constant (k_{cat}) is comparable for I (0.0022 s⁻¹) and III (0.0034 s⁻¹), but is somewhat higher for II (0.0106 s⁻¹). The rate of activation of I by pro-UK is not significantly influenced by the presence of 6-aminohexanoic acid, purified fragments LBS I or LBS II, or histidine-rich glycoprotein, indicating that the high affinity of pro-UK for I is not mediated via the high-affinity LBS I of I located in kringle 1-3 (LBS I) nor via the low-affinity LBS II within kringle 4 (LBS II). Apparently the site(s) in I involved in the high-affinity interaction with pro-UK are located within the III moiety.

IT 82657-92-9

RL: BIOL (Biological study)
(plasminogen of human activation by, of lung adenocarcinoma cells, kinetics of)

=> => fil biosis

FILE 'BIOSIS' ENTERED AT 07:32:13 ON 21 APR 2005

Copyright (c) 2005 The Thomson Corporation

FILE COVERS 1969 TO DATE.

CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 20 April 2005 (20050420/ED)

FILE RELOADED: 19 October 2003.

=> d all tot

L106 ANSWER 1 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
AN 1999:111090 BIOSIS

DN PREV199900111090

TI Production of human prourokinase.

AU Brandazza, A. [Inventor]; Sarmientos, P. [Inventor]; Orsini, G. [Inventor]

CS Rivolta d'Adda, Italy

ASSIGNEE: VASCULAR LABORATORY INC.

PI US 5866358 Feb. 2, 1999

SO Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 2, 1999) Vol. 1219, No. 1, pp. 488. print.
CODEN: OGUPE7. ISSN: 0098-1133.

DT Patent

LA English

ED Entered STN: 12 Mar 1999

Last Updated on STN: 12 Mar 1999

NCL 435069100

CC General biology - Miscellaneous 00532

IT Major Concepts

Biochemistry and Molecular Biophysics; Bioprocess Engineering; Enzymology (Biochemistry and Molecular Biophysics); Infection; Methods and Techniques

IT Miscellaneous Descriptors

BACTERIA; BIOTECHNOLOGY; HUMAN PROUROKINASE PRODUCTION; METHODS

ORGN Classifier

Enterobacteriaceae 06702

Super Taxa

Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria;

Microorganisms

Organism Name

Escherichia coli

Taxa Notes

Bacteria, Eubacteria, Microorganisms

ORGN Classifier

Microorganisms 01000

Super Taxa

Microorganisms

Organism Name

microorganism

Taxa Notes

Microorganisms

L106 ANSWER 2 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
 AN 1997:17614 BIOSIS

DN PREV199799316817

TI A site-directed mutagenesis of **pro-urokinase** which substantially reduces its intrinsic activity.

AU Liu, Jian-Ning [Reprint author]; Tang, Wei; Sun, Zi-Yong; Kung, Wendy; Pannell, Ralph; Sarmientos, Paolo; Gurewich, Victor

CS Vasc. Res. Laboratory, Burlington Build., Room 554B, Deaconess Hosp., One Deaconess Rd., Boston, MA 02215, USA

SO Biochemistry, (1996) Vol. 35, No. 45, pp. 14070-14076.

CODEN: BICHAW. ISSN: 0006-2960.

DT Article

LA English

ED Entered STN: 15 Jan 1997

Last Updated on STN: 23 Jan 1997

AB **Single-chain urokinase-type plasminogen**

activator or **pro-urokinase** is a zymogen with an intrinsic catalytic activity which is greater than that of most other zymogens. To study the structural basis for this activity, a three-dimensional homology model was calculated using the crystallographic structure of chymotrypsinogen, and the structure-function relationship was studied using site-directed mutagenesis and kinetic analysis. This model revealed a unique Lys-300 in **pro-urokinase** which could form a weak interaction with Asp-355, adjacent to the active site Ser-356. It was postulated that this lysine, by its epsilon-amino group, may serve to pull Ser-356 Close to the active position, thereby inducing the higher intrinsic activity of **pro-urokinase**. This was consistent with the published finding that a homologous lysine (Lys-416) in single chain tissue plasminogen activator when mutated to serine induced some reduction in activity. To test this hypothesis, a site-directed mutant with a neutral residue (Lys-300 fwdarw Ala) was produced and characterized. The Ala-300-**pro-urokinase** had a 40-fold lower amidolytic activity than that of **pro-urokinase**. It was also stable in plasma at much higher concentrations than **pro-urokinase**, reflecting much attenuated plasminogen activation. Plasmin activatability was comparable to that of **pro-urokinase**, but the resultant two-chain derivative (Ala-300-**urokinase**) had a lower enzymatic activity (apprxeq 33% that of **urokinase**) due to a reduction of k-cat. Interestingly, the K-M of two-chain Ala-300-**urokinase** against plasminogen was 5.8-fold lower than that of **urokinase**, being similar to that of **pro-urokinase** which has a K-M about 5-fold lower than **urokinase**. In conclusion, the hypothesis that Lys-300 is a key structural determinant of the high intrinsic activity of **pro-urokinase** was confirmed by these studies. This residue also appears to be important for the full expression of the enzymatic activity of **urokinase**.

CC Biochemistry studies - Proteins, peptides and amino acids 10064

Biophysics - Molecular properties and macromolecules 10506

Enzymes - Chemical and physical 10806

Enzymes - Physiological studies 10808

IT Major Concepts

Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and
 Molecular Biophysics)
 IT Chemicals & Biochemicals
 PRO-UROKINASE; SERINE PROTEASE
 IT Miscellaneous Descriptors
 ANALYTICAL METHOD; COMPARISON; ENZYMOLOGY; PRO-
 UROKINASE; SERINE PROTEASE COMPARISON; SITE-DIRECTED
 MUTAGENESIS; STRUCTURE-ACTIVITY RELATIONSHIP; THREE-DIMENSIONAL
 STRUCTURE; ZYMOGEN
 RN 82657-92-9 (PRO-UROKINASE)
 37259-58-8 (SERINE PROTEASE)

L106 ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
 AN 1993:432624 BIOSIS
 DN PREV199396087249
 TI Implication of cystein residues in the activity of single-
 chain urokinase-plasminogen activator.
 AU Hamelin, Jocelyne [Reprint author]; Sarmientos, Paolo; Orsini,
 Gaetano; Galibert, Francis
 CS Lab. de Recombinaisons Genetiques, Centre Hayem, Hopital Saint-Louis,
 75475, Paris Cedex 10, France
 SO Biochemical and Biophysical Research Communications, (1993) Vol. 194, No.
 2, pp. 978-985.
 CODEN: BBRCA9. ISSN: 0006-291X.
 DT Article
 LA English
 ED Entered STN: 22 Sep 1993
 Last Updated on STN: 23 Sep 1993
 AB Single-chain urokinase-plasminogen activator
 contains 24 cysteine residues involved in 12 disulfide bonds and
 distributed all along the three domains of the protein. In order to
 investigate the role of these disulfide bridges in the catalytic
 activities of scu-PA, we used site-specific
 mutagenesis to construct 10 mutants in which some cysteine residues were
 changed to serine residues. Each mutated DNA fragment was cloned into a
 procaryotic expression vector and the protein expressed in E. coli.
 Mutant proteins of the expected size were produced and analyzed for
 amidolytic and fibrinolytic activities. From this, it is shown that: i)
 the disulfide bonds in the epidermal growth factor (EGF)-like and in the
 kringle domains are not necessary. Moreover, disulfide bond deletion in
 the kringle domain improves those catalytic activities; ii) on the
 contrary, the disulfide bridges in the catalytic domain are essential for
 maintaining both activities.

CC Genetics - General 03502
 Biochemistry studies - Nucleic acids, purines and pyrimidines 10062
 Biochemistry studies - Proteins, peptides and amino acids 10064
 Biophysics - Methods and techniques 10504
 Biophysics - Molecular properties and macromolecules 10506
 Enzymes - Chemical and physical 10806
 Enzymes - Physiological studies 10808
 Metabolism - Nucleic acids, purines and pyrimidines 13014
 Blood - Blood and lymph studies 15002

IT Major Concepts
 Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and
 Molecular Biophysics); Genetics; Metabolism; Methods and Techniques
 IT Chemicals & Biochemicals
 CYSTEIN
 IT Miscellaneous Descriptors
 CARCINOGENESIS; DEVELOPMENTAL REGULATION; HUMAN GASTRIC CANCER
 COMPLEMENTARY DNA; NORTHERN BLOT ANALYSIS; RAT EMBRYO STOMACH

ORGN Classifier
 Animalia 33000
 Super Taxa

Animalia
 Organism Name
 Animalia
 Taxa Notes
 Animals
 RN 52-90-4 (CYSTEIN)

L106 ANSWER 4 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
 AN 1992:408842 BIOSIS
 DN PREV199294072042; BA94:72042
 TI STRUCTURE-FUNCTION RELATIONSHIP OF BASIC FIBROBLAST GROWTH FACTOR
 SITE-DIRECTED MUTAGENESIS OF A PUTATIVE HEPARIN-BINDING AND
 RECEPTOR-REGION.
 AU PRESTA M [Reprint author]; STATUTO M; ISACCHI A; CACCIA P; POZZI A;
 GUALANDRIS A; RUSNATI M; BERGONZONI L; SARMIENTOS P
 CS UNIT GENERAL PATHOL AND IMMUNOL, DEP BIOMEDICAL SCIENCES AND BIOTECHNOL,
 SCH MED, UNIV BRESCIA, 25123 BRESCIA, ITALY
 SO Biochemical and Biophysical Research Communications, (1992) Vol. 185, No.
 3, pp. 1098-1107.
 CODEN: BBRCA9. ISSN: 0006-291X.
 DT Article
 FS BA
 LA ENGLISH
 ED Entered STN: 9 Sep 1992
 Last Updated on STN: 9 Sep 1992
 AB Basic residues Arg-118, Lys-119, Lys-128, and Arg-129 within a putative heparin-binding and receptor-binding region of the 155-amino acid form of basic fibroblast growth factor (bFGF) have been changed to neutral glutamine residues by site-directed mutagenesis of the human bFGF cDNA. The bFGF mutant (M6B-bFGF) was expressed in *E. coli* and purified to homogeneity. When compared to wild type bFGF, M6B-bFGF showed in cultured endothelial cells a similar receptor-binding capacity and mitogenic activity, but a reduced affinity for heparin-like low affinity binding sites, a reduced chemotactic activity, and a reduced capacity to induce the production of urokinase-type plasminogen activator. In vivo, M6B-bFGF lacked a significant angiogenic activity. Modifications of both the primary and the tertiary structure of bFGF appear to be responsible for the modified biological properties of M6B-bFGF, thus confirming the possibility to dissociate at the structural level some of the biological activities exerted by BFGF on endothelial cells.
 CC Cytology - Human 02508
 Biochemistry studies - Nucleic acids, purines and pyrimidines 10062
 Biochemistry studies - Proteins, peptides and amino acids 10064
 Biochemistry studies - Carbohydrates 10068
 Cardiovascular system - Physiology and biochemistry 14504
 Endocrine - General 17002
 Physiology and biochemistry of bacteria 31000
 IT Major Concepts
 Biochemistry and Molecular Biophysics; Cardiovascular System (Transport and Circulation); Cell Biology; Endocrine System (Chemical Coordination and Homeostasis); Physiology
 IT Miscellaneous Descriptors
 ESCHERICHIA-COLI HUMAN ENDOTHELIAL CELLS ARGININE-118 LYSINE-119
 LYSINE-128 ARGININE-129 COMPLEMENTARY DNA
 ORGN Classifier
 Enterobacteriaceae 06702
 Super Taxa
 Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria;
 Microorganisms
 Taxa Notes
 Bacteria, Eubacteria, Microorganisms
 ORGN Classifier
 Hominidae 86215

Super Taxa

Primates; Mammalia; Vertebrata; Chordata; Animalia

Taxa Notes

Animals, Chordates, Humans, Mammals, Primates, Vertebrates

L106 ANSWER 5 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
 AN 1991:501012 BIOSIS
 DN PREV199192123972; BA92:123972
 TI USE OF THE UROKINASE-TYPE PLASMINOGEN ACTIVATOR GENE AS A
 GENERAL TOOL TO MONITOR EXPRESSION IN TRANSGENIC ANIMALS STUDY OF THE
 TISSUE-SPECIFICITY OF THE MURINE WHEY ACIDIC PROTEIN WAP EXPRESSION
 SIGNALS.
 AU BRANDAZZA A [Reprint author]; LEE E; FERRERA M; TILLMAN U; SARMIENTOS
 P; WESTPHAL H
 CS FARMITALIA, CARLO ERBA, DEP BIOTECHNOLOGY, VIALE BEZZI 24, 20146 MILANO,
 ITALY
 SO Journal of Biotechnology, (1991) Vol. 20, No. 2, pp. 201-212.
 CODEN: JBITD4. ISSN: 0168-1656.
 DT Article
 FS BA
 LA ENGLISH
 ED Entered STN: 12 Nov 1991
 Last Updated on STN: 13 Nov 1991
 AB Urokinase-type plasminogen activator (uPA) is a proteolytic
 enzyme able to convert the zymogen plasminogen into the strong protease
 plasmin. The availability of very sensitive tests to measure the
 enzymatic activity of a plasminogen activator renders the corresponding
 gene an ideal candidate for the detection of promoter activity. In this
 paper we describe the utilization of the human uPA gene as detector of
 tissue-specificity of the murine whey acidic protein (WAP) expression
 signals in transgenic mice. The WAP promoter has been previously
 investigated for the production of foreign proteins in the milk of
 transgenic animals. In our genetic constructions, the human uPA cDNA was
 linked to the promoter region as well as to 3'-end distal sequences of the
 WAP gene. Five transgenic lines were obtained in which, however,
 expression levels of human uPA in the milk were still quite low.
 Surprisingly, four of these five positive transgenic mice show a
 consistent activity of the WAP promoter in brain extracts compared to
 other tissues.
 CC Genetics - General 03502
 Genetics - Animal 03506
 Genetics - Human 03508
 Comparative biochemistry 10010
 Biochemistry methods - General 10050
 Biochemistry methods - Nucleic acids, purines and pyrimidines 10052
 Biochemistry methods - Proteins, peptides and amino acids 10054
 Biochemistry studies - General 10060
 Biochemistry studies - Nucleic acids, purines and pyrimidines 10062
 Biochemistry studies - Proteins, peptides and amino acids 10064
 Biophysics - Molecular properties and macromolecules 10506
 Enzymes - Methods 10804
 Enzymes - Chemical and physical 10806
 Enzymes - Physiological studies 10808
 Physiology - General 12002
 Metabolism - General metabolism and metabolic pathways 13002
 Metabolism - Proteins, peptides and amino acids 13012
 Metabolism - Nucleic acids, purines and pyrimidines 13014
 Blood - General and methods 15001
 Blood - Blood and lymph studies 15002
 Reproductive system - Physiology and biochemistry 16504
 Nervous system - Physiology and biochemistry 20504
 Laboratory animals - General 28002
 IT Major Concepts

Animal Care; Biochemistry and Molecular Biophysics; Blood and Lymphatics (Transport and Circulation); Enzymology (Biochemistry and Molecular Biophysics); Genetics; Metabolism; Nervous System (Neural Coordination); Reproductive System (Reproduction)

IT Miscellaneous Descriptors

HUMAN GENE COMPARISON ENZYMATIC ACTIVITY MILK PROMOTER METHOD GENETIC ENGINEERING

ORGN Classifier

Hominidae 86215

Super Taxa

Primates; Mammalia; Vertebrata; Chordata; Animalia

Taxa Notes

Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier

Muridae 86375

Super Taxa

Rodentia; Mammalia; Vertebrata; Chordata; Animalia

Taxa Notes

Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 9039-53-6 (UROKINASE)

L106 ANSWER 6 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
AN 1991:248288 BIOSIS

DN PREV199191128843; BA91:128843

TI A SIX-AMINO ACID DELETION IN BASIC FIBROBLAST GROWTH FACTOR DISSOCIATES ITS MITOGENIC ACTIVITY FROM ITS PLASMINOGEN ACTIVATOR-INDUCING CAPACITY.

AU ISACCHI A [Reprint author]; STATUTO M; CHIESA R; BERGONZONI L; RUSNATI M; SARMIENTOS P; RAGNOTTI G; PRESTA M

CS GENERAL PATHOL, DEP BIOMEDICAL SCI, SCH MED, UNIV BRESCIA, VIA VALSABBINA 19, 25133 BRESCIA, ITALY

SO Proceedings of the National Academy of Sciences of the United States of America, (1991) Vol. 88, No. 7, pp. 2628-2632.

CODEN: PNASA6. ISSN: 0027-8424.

DT Article

FS BA

LA ENGLISH

ED Entered STN: 25 May 1991

Last Updated on STN: 25 May 1991

AB A recombinant deletion mutant of the 155-amino acid form of human basic fibroblast growth factor (bFGF), lacking amino acid residues 27-32 (Lys-Asp-Pro-Lys-Arg-Leu), was expressed in *Escherichia coli* and purified to homogeneity by heparin-Sepharose affinity chromatography. When maintained in the presence of an equimolar concentration of soluble heparin, the bFGF mutant (M1-bFGF) is as potent as bFGF in stimulating cell proliferation in normal and transformed fetal bovine aortic endothelial cells, in adult bovine aortic endothelial cells, and in NIH 3T3 fibroblasts. However, under the same experimental conditions, M1-bFGF is at least 100 times less efficient than bFGF in stimulating plasminogen activator (PA) production in endothelial cells, as assayed by chromogenic PA assay, SDS/PAGE zymography, and Northern blot analysis of urokinase-type PA mRNA. In the presence of heparin, M1-bFGF binds to bFGF plasma membrane receptors present on endothelial cells in a manner undistinguishable from bFGF. It also induces the same tyrosine phosphorylation pattern when added to NIH 3T3 cells. The data suggest that the PA-inducing activity of bFGF may depend upon a functional domain that differs from those involved in the mitogenic activity of the growth factor and that the binding of bFGF to its plasma membrane receptor may not be sufficient to induce urokinase-type PA production in endothelial cells.

CC Cytology - Animal 02506

Biochemistry studies - Proteins, peptides and amino acids 10064

Biochemistry studies - Carbohydrates 10068

Biophysics - Molecular properties and macromolecules 10506
 Cardiovascular system - Physiology and biochemistry 14504
 Endocrine - General 17002

IT Major Concepts
 Biochemistry and Molecular Biophysics; Cardiovascular System (Transport and Circulation); Cell Biology; Endocrine System (Chemical Coordination and Homeostasis)

IT Miscellaneous Descriptors
 HUMAN 3T3 CELL BOVINE ENDOTHELIAL CELL RECEPTOR STRUCTURE FUNCTION

ORGN Classifier
 Bovidae 85715

Super Taxa
 Artiodactyla; Mammalia; Vertebrata; Chordata; Animalia

Taxa Notes
 Animals, Artiodactyls, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Vertebrates

ORGN Classifier
 Hominidae 86215
 Super Taxa
 Primates; Mammalia; Vertebrata; Chordata; Animalia

Taxa Notes
 Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier
 Muridae 86375
 Super Taxa
 Rodentia; Mammalia; Vertebrata; Chordata; Animalia

Taxa Notes
 Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

L106 ANSWER 7 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
 AN 1991:230709 BIOSIS

DN PREV199191122169; BA91:122169

TI EFFICIENT RENATURATION AND FIBRINOLYTIC PROPERTIES OF PROUROKINASE AND A DELETION MUTANT EXPRESSED IN ESCHERICHIA-COLI AS INCLUSION BODIES.

AU ORSINI G [Reprint author]; BRANDAZZA A; SARMIENTOS P; MOLINARI A; LANSEN J; CAUET G

CS FARMITALIA, DEP BIOTECHNOL, VIALE BEZZI 24, I-20146 MILANO, ITALY

SO European Journal of Biochemistry, (1991) Vol. 195, No. 3, pp. 691-698.
 CODEN: EJBCAI. ISSN: 0014-2956.

DT Article

FS BA

LA ENGLISH

ED Entered STN: 9 May 1991

Last Updated on STN: 10 May 1991

AB Prourokinase is a plasminogen activator of 411 amino acids which displays a clot-lysis activity through a fibrin-dependent mechanism, and which seems to be a promising agent for the treatment of acute myocardial infarction. The preparation of recombinant prourokinase in bacteria has been hampered by its insolubility and by difficulty in refolding in the polypeptide chain. In this paper we describe the renaturation process of two recombinant proteins expressed in *Escherichia coli* as inclusion bodies: prourokinase and a deletion derivative Δ 125- prourokinase and 0.25 h-1 for prourokinase). Our process involves sequential steps of denaturation, reduction and controlled refolding of the polypeptide chain. When applied to pure, non-glycosylated and active prourokinase, it gives a refolding yield of about 80%, demonstrating the efficiency of the renaturation procedure. Lower yields (15% and 30%), respectively, for prourokinase and Δ 125- prourokinase) were obtained when the same refolding protocol was applied to inclusion bodies from bacteria. After purification to homogeneity (as shown by HPLC and SDS/PAGE) specific activities were 160,000 and 250,000 IU/mg protein,

respectively, for **prourokinase** and $\Delta 125$ -**prourokinase**. As with **prourokinase**, the deletion mutant $\Delta 125$ - **prourokinase** displays a zymogenic nature, being activation by plasmin to the active two-chain form; however, this mutant is approximately fourfold more resistant than **prourokinase** to plasmin activation, and consequently shows a different fibrinolytic profile.

CC Biochemistry studies - Proteins, peptides and amino acids 10064
 Enzymes - Chemical and physical 10806
 Blood - Blood and lymph studies 15002
 Pharmacology - Blood and hematopoietic agents 22008
 Physiology and biochemistry of bacteria 31000
 Genetics of bacteria and viruses 31500
 Food microbiology - Antibiotics, biologics and other agents 39004
 IT Major Concepts
 Biochemistry and Molecular Biophysics; Blood and Lymphatics (Transport and Circulation); Enzymology (Biochemistry and Molecular Biophysics); Pharmacology
 IT Miscellaneous Descriptors
 RECOMBINANT PROTEINS GENETICALLY-ENGINEERED PROTEINS THROMBOLYTIC AGENTS PROTEIN FOLDING
 ORGN Classifier
 Enterobacteriaceae 06702
 Super Taxa
 Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria;
 Microorganisms
 Taxa Notes
 Bacteria, Eubacteria, Microorganisms
 RN 82657-92-9 (PROUROKINASE)

=> d his

(FILE 'HOME' ENTERED AT 06:51:51 ON 21 APR 2005)
 DEL HIS

FILE 'HCAPLUS' ENTERED AT 06:53:57 ON 21 APR 2005

L1 712 S ?PROUK? OR ?PROUROKINASE? OR PRO() (UK OR UROKINASE)
 L2 3 S RPRO() (UK OR UROKINASE)
 L3 712 S L1,L2

FILE 'REGISTRY' ENTERED AT 06:55:45 ON 21 APR 2005

L4 1 S 82657-92-9
 E PROUROKINASE
 L5 151 S E3

FILE 'HCAPLUS' ENTERED AT 06:57:31 ON 21 APR 2005

L6 684 S L4
 L7 714 S L5
 L8 545 S ABT 187 OR ABT187 OR PRO U PA OR PROLYSE OR PROLYZE OR PUK OR
 L9 1171 S L3,L6-L8
 L10 7 S PET29A
 L11 20 S ?PET29?
 L12 4 S L9 AND L10,L11
 L13 9 S L9 AND T7
 L14 6 S L9 AND (SHINE OR DALGARNO)
 L15 16 S L9 AND (BL21 OR DE3 OR RIL)
 L16 7 S L12-L14 AND L15
 E E COLI/CT
 E ESCHERICHIA/CT
 L17 148991 S E3+OLD,NT,PFT,RT OR E14+OLD,NT,PFT,RT
 L18 248421 S ("E" OR ESCHERICH?) ()COLI
 L19 176 S L9 AND L17,L18

L20 2 S L19 AND TYPE B
 L21 108 S L9 AND B
 L22 3 S L20,L21 AND L10-L16
 L23 153 S L9 AND ESCHERI?
 L24 21 S L23 AND L10-L16
 L25 3 S L24 AND (TYPE B OR B)
 L26 27 S L12-L16,L20,L22,L25
 L27 235 S L19,L21,L23,L24 NOT L26
 L28 4 S L26 AND ?MUTANT?
 L29 2 S L26 AND ?MUTAT?
 L30 36 S L27 AND (?MUTANT? OR MUTAT?)
 L31 2 S L26 AND ?MUTAGEN?
 L32 15 S L27 AND ?MUTAGEN?
 L33 4 S L28,L29,L31
 L34 41 S L30,L32 NOT L33
 L35 2 S L9 AND FLEX? (L) LOOP?
 L36 1 S L9 AND WOBBL? (L) LOOP?
 L37 2 S L35,L36
 L38 6 S L9 AND (LYS300 OR LYS 300)
 L39 0 S L9 AND (LYSINE300 OR LYSINE 300)
 L40 27 S L9 AND (HIS OR HISTID?)
 L41 124 S L9 AND (LYS OR LYSINE OR LYSYL?)
 L42 12 S L40 AND L41

FILE 'REGISTRY' ENTERED AT 07:06:40 ON 21 APR 2005

L43 1 S 56-87-1
 L44 3 S (D-LYSINE OR DL-LYSINE)/CN OR L43
 L45 3 S (L-HISTIDINE OR D-HISTIDINE OR DL-HISTIDINE) /CN

FILE 'HCAPLUS' ENTERED AT 07:07:20 ON 21 APR 2005

L46 24 S L44 AND L9
 L47 6 S L45 AND L9
 L48 13 S L38,L41,L46 AND L40,L47
 L49 2 S L48 AND L10-L16,L26
 L50 4 S L48 AND L27
 L51 3 S L48 AND L28-L37
 L52 6 S L49-L51
 L53 5 S L52 NOT CRYOGEL/TI
 L54 96 S L10-L16,L20,L22,L25,L26,L28-L37,L48
 L55 5 S L54 AND L53
 L56 4 S L55 NOT MMP 3/TI
 L57 91 S L54 NOT L55
 L58 15 S L57 AND PROUROKINASE/TI
 L59 19 S L56,L58
 L60 76 S L57 NOT L59
 L61 35 S L60 AND L6
 L62 38 S L60 AND L7
 L63 57 S L61,L62,L59
 L64 38 S L54 NOT L55-L56,L58,L59,L61-L63
 SEL DN AN 13-16 22 24 26 29-34 36-38
 L65 16 S L64 AND E1-E48
 L66 73 S L63,L65
 L67 72 S L66 AND ?UROKINASE?
 L68 1 S L66 NOT L67
 E SARMIENTOS P/AU
 L69 46 S E3,E4
 E PAGANI M/AU
 L70 45 S E3-E8,E18
 L71 9 S L69,L70 AND L9
 L72 12 S L69,L70 AND ?UROKINASE?
 L73 12 S L71,L72
 L74 2 S US20050019863/PN OR (WO2004-US11840 OR US2004-826598# OR US20
 L75 1 S L74 AND L9

L76 1 S L74 AND ?UROKINASE?
 L77 12 S L75, L76, L73
 L78 78 S L77, L67
 L79 78 S L78 AND L1-L3, L6-L42, L46-L78
 L80 75 S L79 AND (PD<=20030418 OR PRD<=20030418 OR AD<=20030418)
 L81 3 S L79 NOT L80
 L82 78 S L79-L80
 L83 49 S L82 AND (?MUTANT? OR ?MUTAGEN? OR ?MUTAT?)
 E MUTANT/CT
 E MUTAT/CT
 L84 332100 S E7+OLD, NT, PFT, RT
 E MUTAGEN/CT
 L85 202397 S E5+OLD, NT, PFT, RT OR E5-E10
 L86 268465 S E16+OLD, NT, PFT, RT
 L87 13 S L82 AND L84-L86
 L88 50 S L83, L87
 L89 45 S L82 AND (RECOMBIN? OR CHIMER?)
 E RECOMBINANT/CT
 L90 0 S L82 AND E11+OLD, NT, PFT, RT
 L91 0 S L82 AND E41+OLD, NT, PFT, RT
 L92 28 S L82 AND E48+OLD, NT, PFT, RT
 E E48+ALL
 L93 28 S L82 AND E9+OLD, NT, PFT, RT
 L94 4 S L82 AND E7+OLD, NT, PFT, RT
 L95 70 S L88-L94
 L96 8 S L82 NOT L95
 SEL DN AN 1 4 5
 L97 5 S L96 NOT E1-E9
 L98 75 S L95, L97

FILE 'REGISTRY' ENTERED AT 07:29:12 ON 21 APR 2005

FILE 'HCAPLUS' ENTERED AT 07:29:23 ON 21 APR 2005

FILE 'BIOSIS' ENTERED AT 07:30:00 ON 21 APR 2005
 E SARMIENTOS P/AU

L99 39 S E3, E4
 E PAGANI M/AU
 L100 269 S E3-E11, E21
 L101 1043 S L1, L2, L4, L5, L8
 L102 12623 S ?UROKINASE?
 L103 7 S L99, L100 AND L101, L102
 L104 7 S L103 AND PY<=2003
 L105 1 S L103 AND P/DT
 L106 7 S L104, L105

FILE 'BIOSIS' ENTERED AT 07:32:13 ON 21 APR 2005

=>